# Relaxation of Water Protons in Highly Concentrated Aqueous Protein Systems Studied by $^1$H NMR Spectroscopy

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Concentrated Aqueous Protein Systems, Proton Relaxation Times, Slow Chemical Exchange

In this paper we present proton spin-lattice ($T_1$) and spin-spin ($T_2$) relaxation times measured vs. concentration, temperature, pulse interval ($\tau_{CPMG}$) as well as $^1$H NMR spectral measurements in a wide range of concentrations of bovine serum albumin (*BSA*) solutions. The anomalous relaxation behaviour of the water protons, similar to that observed in mammalian lenses, was found in the two most concentrated solutions (44% and 46%). The functional dependence of the spin-spin relaxation time vs. $\tau_{CPMG}$ pulse interval and the values of the motional activation parameters obtained from the temperature dependencies of spin-lattice relaxation times suggest that the water molecule mobility is reduced in these systems. The slow exchange process on the $T_2$ time scale is proposed to explain the obtained data. The proton spectral measurements support the hypothesis of a slow exchange mechanism in the highest concentrated solutions. From the analysis of the shape of the proton spectra the mean exchange times between *bound* and *bulk* water proton groups ($\tau_{ex}$) have been estimated for the range of the highest concentrations (30%–46%). The obtained values are of the order of milliseconds assuring that the slow exchange condition is fulfilled in the most concentrated samples.

## Introduction

In general, protons in every protein-water system may be classified into at least three main groups: the freezable *bulk water protons* ($A$) – not interacting with the protein, the nonfreezable *bound water protons* ($B$) in the hydration layer of the protein and the *protein protons* ($C$) e.g. the protons in the external, functional groups on the surface of the protein macromolecules. The dynamic behaviour of *bulk* water molecules is assumed to be isotropic and is similar to that observed in pure water (Blicharska *et al.*, 1970). The mobility of *bound* water molecules is significantly reduced as a result of its hydrogen bonding to the protein surface (Kimmich *et al.*, 1990). The mobility of the protein protons (group $C$) is determined by the reorientation of protein macromolecules and the relaxation times of protein protons are much shorter than observed for water protons.

Two possible mechanisms of exchange may exist between the mentioned proton groups. The first is the material exchange, which occurs mainly between the *bulk* and *bound* water protons. The process is fast when the mean residence times of protons are significantly shorter than its relaxation times. In this case monoexponential relaxation decays with medium (average) relaxation rates are expected (Daszkiewicz *et al.*, 1963). In the slow or moderately slow exchange limits, multiexponential relaxation curves and several distinguishable values of the relaxation times are measured (Winkler and Michel, 1985). The second exchange mechanism is the immaterial spin exchange, termed cross-relaxation or, in the case of secular dipolar broadening, spin diffusion (Kimmich *et al.*, 1990; 1993; Koenig *et al.*, 1993). When this process controls the relaxation behaviour of the system, both, spin-lattice and spin-spin relaxation curves are nonexponential (Edzes and Samulski, 1978; Gutsze *et al.*, 1995). However, in most biological systems the exchange of spin magnetisation was considered to occur at faster rate than spin-lattice relaxation of protons (Bodurka *et al.*, 1999; Sykes *et al.*, 1978; Kalk and Berendsen, 1976). Under such conditions the cross-relaxation term may be

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included to the relaxation rate of the faster relaxing *bound* water protons.

This simple model has been used to explain the relaxation behaviour of the protons in low concentrated protein solutions (Blicharska *et al.*, 1970; Lammana *et al.*, 1989; Blinc *et al.*, 1990). In such protein-water systems, where the protein weight (w/w) concentration do not exceed 30%, single (medium) values of relaxation times are observed, indicating that the proton exchange mechanisms are fast in the relaxation time scale. Additionally, the values of both relaxation times are approximately equal (the ratio $R = T_1/T_2$ is close to 1) and decrease when the protein concentration is increased (Daszkiewicz *et al.*, 1963).

Unusual relaxation behaviour was detected in mammalian lenses where the mean concentration of the protein is extremely high (~ 35%). In such systems the proton relaxation time values are significantly shorter than that measured in pure water as a result of anisotropic motion of water molecules (Bodurka *et al.*, 1995; Bodurka *et al.*, 1996b). The ratio $R = T_1/T_2$ exceeds unity, i.e. $T_{2A}$, $T_{2B} \ll T_1$, indicating a solid-like behaviour of the water in these systems. Further difference is presence of the biexponential spin-spin relaxation decay. To explain these results the assumption of the slow exchange mechanism in such systems is necessary (Gutsze *et al.*, 1995; Bodurka *et al.*, 1996a; Bodurka *et al.,* 1997). The proton exchange mechanisms are fast enough to average spin-lattice but not spin-spin relaxation processes in *bulk* and *bound* water proton groups.

Purpose of this paper was to examine and compare the relaxation behaviour of the lens and its model system – concentrated protein solution. In particular it was important to establish if the anomalous water behaviour observed in the lenses is either their unique characteristic or may be found in another kind of high concentrated protein-water system – protein solution. Results of different relaxation and proton spectral measurements in the wide concentration range of bovine serum albumin (*BSA*) solutions presented in this contribution allowed us to elucidate this problem in a sufficient way.

## Materials and Methods

Measurements of spin-spin and spin-lattice relaxation times were performed with the bovine serum albumin (*BSA*, Fraction V, supplied by Fluka Chemie AG, Buchs, mw ~ 67 kDa) solutions, dissolved in Tris(hydroxymethyl)aminomethane-HCl buffer with physiological pH of 7.4. All the solutions were prepared by adding the proper amount of the protein to the buffer. The investigated protein weight (w/w) concentrations, $c$, (in grams per 1 gram of solution) cover the range from 0% (pure solvent) to 46% w/w *BSA*. All the measurements were performed within ten hours after preparation the solution.

The proton relaxation measurements were carried out on the PMS-60 pulse spectrometer (Radiopan, Poznan, Poland) working at the frequency of 60 MHz. The measurements of the proton spectral lines in the solutions were performed on the AM 270 SY spectrometer working at 270 MHz (Bruker, Germany).

Spin-spin relaxation times ($T_2$) were measured with the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence: $\pi/2_x$, $(\tau_{CPMG}, \pi_y, \tau_{CPMG})_n$. Only even echoes were registered to reduce the effects of the $B_1$ field inhomogeneity and pulse width errors (Levitt and Freeman, 1969). The value of the pulse spacing time ($\tau_{CPMG}$) varied from 60 μs to 6 ms. The measurements of spin-lattice relaxation time were performed using the Inversion Recovery (IR) method with the pulse sequence: $\pi_x$, $\tau_{IR}$, $\pi/2_x$. The relaxation functions were analysed by the non-linear least square method (Marquart algorithm). The main part of the measurements was done at room temperature (295 K). In the $T_1$ vs. temperature ($T$) measurements the temperature of the sample was stabilised with the accuracy of 1 K. The deviations of the obtained relaxation times were below 10% for samples with the same protein content.

## Results and Discussion

### *The relaxation rates as a function of the protein concentration*

Representative values of $T_1$ and $T_2$ relaxation times measured for various *BSA* w/w concentrations are listed in Table I. Both relaxation times decreases when the weight concentration of protein in the solution increases. For the whole concentration range spin-lattice relaxation curves were monoexponential. Thus, we conclude that the two-phase model of material exchange between *A*

Table I. Spin-lattice ($T_1$) and spin-spin ($T_2$, $T_{2A}$, $T_{2B}$) relaxation times for selected *BSA* solutions at the concentration range from 0% (pure solvent) to 46%, compared with the $\tau_{ex}$ (mean exchange time) values between the *bound* and *bulk* water proton groups determined from the spectral measurements.

| *BSA* concentration [% w/w] | $T_1$ | $T_2$ | $T_{2A}$ | $T_{2B}$ | $\tau_{ex}$ |
|---|---|---|---|---|---|
| | | | [ms] | | |
| 0% | 2281 ± 5 | 2230 ± 5 | – | – | – |
| 10% | 1270 ± 3 | 507 ± 1 | – | – | – |
| 20% | 821 ± 1 | 243 ± 1 | – | – | – |
| 30% | 576 ± 1 | 83 ± 1 | – | – | 6.4 ± 0.4 |
| 40% | 443 ± 1 | 25.7 ± 0.3 | – | – | 7.3 ± 0.7 |
| 42% | 400 ± 1 | 15.4 ± 0.2 | – | – | 7.6 ± 0.5 |
| 44% | 381 ± 1 | – | 13.2 ± 0.7 | 7.6 ± 1.5 | 7.6 ± 0.2 |
| 46% | 359 ± 1 | – | 12.2 ± 1.8 | 5.8 ± 0.7 | 7.4 ± 0.2 |

and *B* proton groups may be applied in every case investigated here. Monoexponential spin-spin relaxation decays were observed only in the range of protein concentrations from 0% (pure solvent) to 42%. For the most concentrated systems, i.e. 44% and 46% of *BSA*, $T_2$ relaxation functions were biexponential and, in consequence, two distinct values of spin-spin relaxation time: $T_{2A}$ and $T_{2B}$, were measured. Furthermore, spin-lattice relaxation times (order of seconds) were much longer than spin-spin relaxation times (order of milliseconds). Experimental results show that the slow material exchange process, on $T_2$ time scale is observed in two highest concentrated solutions. It is probably caused by significant water molecules mobility restrictions present in such systems. Such restrictions may cause the anisotropic reorientation of water molecules, which could be detected in the CPMG experiment (Cohen-Addad and Vogin, 1974).

### *$T_2$ relaxation rates as a function of the pulse interval, $\tau_{CPMG}$*

The chemical shifts of *bulk* and *bound* water differ from each other, what should be taken into account in the analysis of $T_2$ relaxation rates on the pulse interval, $\tau_{CPMG}$, dependencies (Allerhand and Gutowski, 1965). In the case of chemical exchange between two groups of protons (*A* and *B*) with different chemical shifts, effective (observed in the experiment) spin-spin relaxation rate dependence on the pulse interval is given by the following equation (Luz and Meiboom, 1963):

$$\frac{1}{T_2} = \frac{1}{T_{2m}} + p_A p_B (\delta\omega_{AB})^2 \tau_{ex} \cdot \left[1 - \left(\frac{2\tau_{ex}}{\tau_{CPMG}}\right) tanh\left(\frac{\tau_{CPMG}}{2\tau_{ex}}\right)\right], \quad (1)$$

where: $1/T_{2m}$ is the average spin-spin relaxation rate in the presence of fast chemical exchange between the spin fractions $p_A$ and $p_B$, $\delta\omega_{AB}$ is the difference between the proton resonance frequencies (chemical shifts) in both groups, and $\tau_{ex}$ is the mean exchange time between the groups, given by the following expression:

$$\tau_{ex} = \frac{\tau_A \cdot \tau_B}{\tau_A + \tau_B}, \quad (2)$$

where: $\tau_A$, $\tau_B$ are the mean residence times in the groups *A* and *B* respectively.

Representative results of $1/T_2$ ($\tau_{CPMG}$) measurements performed on 30%, 40% and 46% solutions are shown in Figs. 1 and 2. The expression (1) fits to the experimental data obtained for low concentrated solutions (≤30%). The example of this fitting to 30% solution data is shown in Fig. 1 as a solid line. For more concentrated samples the mean exchange times are longer than the time intervals used in the CPMG experiment. Therefore, the increase of the relaxation rate for the longest $\tau_{CPMG}$ cannot be expected. Indeed, for 40% and 46% solutions measured relaxation rates do not exhibit any increase for the long $\tau_{CPMG}$. The relaxation rates, however, increase in the region of short $\tau_{CPMG}$ interval, which is not predicted by the Luz-Meiboom expression. Additionally, for this probe the relaxation decay is biexponential in the whole pulse interval range. The differences in the experimental data of low and high concentrated

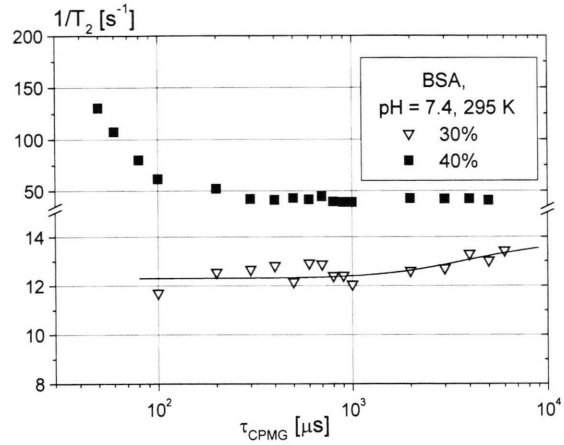


Fig. 1. Spin-spin relaxation rates dependencies on the pulse interval ($\tau_{CPMG}$) for *BSA* concentrations: 30% and 40%. Solid line represents the fit of the Luz-Meiboom expression (1) to the experimental results of 30% solution.

solutions can be interpreted via an existence of non-zero time averaging of the spin pair dipolar energy within the water molecule (Ahmad *et al.*, 1977). It is a result of the motional restrictions leading to the water anisotropic reorientation (Cohen-Addad, 1974). Similar explanation was previously proposed for mammalian lenses (Bodurka *et al.*, 1995).

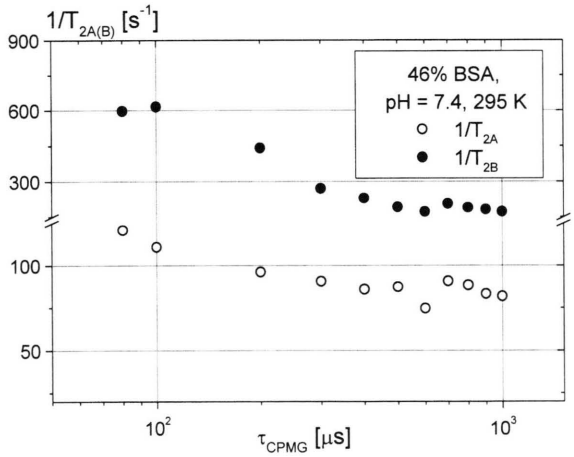


Fig. 2. Spin-spin relaxation rates dependencies on the pulse interval ($\tau_{CPMG}$) for 46% *BSA* concentration.

*$T_1$ relaxation rates as a function of temperature*

The reduction of the motional ability of the water molecules should also lead to the increase of the motional activation parameters (activation energy, $E_A$ and correlation time, $\tau_c$). Its values can be calculated from the spin-lattice relaxation times dependencies on the temperature, according to the simple model of magnetic dipole interactions, using the Bloembergen formula:

$$\frac{1}{T_1} = \frac{2}{3} \cdot \gamma^2 \cdot \langle \Delta H^2 \rangle \cdot \left[ \frac{\tau_c}{1 + (\omega\tau_c)^2} + \frac{4\tau_c}{1 + (2\omega\tau_c)^2} \right], \quad (3)$$

where: $\langle \Delta H^2 \rangle$ is the second moment and the correlation time, $\tau_c$, is given by the Arrhenius equation:

$$\tau_c = \tau_{c0} \exp\left(\frac{E_A}{RT}\right), \quad (4)$$

where: $T$ is the temperature and $\tau_{c0}$ is the asymptotic value of $\tau_c$ for $T \rightarrow \infty$.

The values of motional activation parameters obtained from the fitting of Eqns. (3) and (4) to the experimental data of 35%, 40% and 45% solutions are listed in Table II. Fig. 3 shows the example of this fitting to 35% solution data, represen-

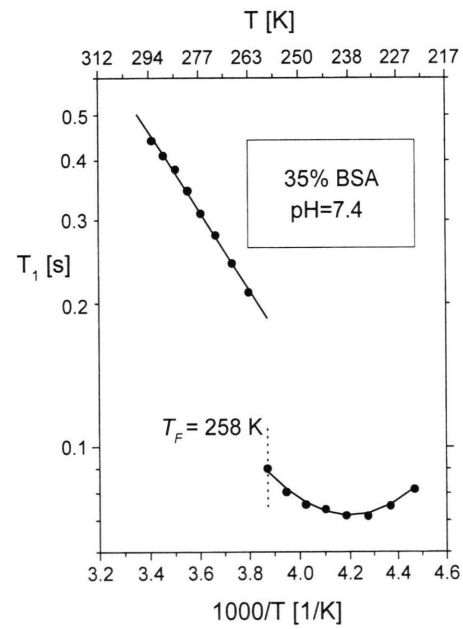


Fig. 3. Temperature dependence of $T_1$ relaxation times for 35% *BSA* solution. A discontinuity at the temperature $T_F$ is connected with the freezing process of the *bulk* water. Solid lines result from fitting of Eqns. (3) and (4) to the experimental data.

Table II. Activation parameters of molecular motions calculated on the base of magnetic dipole interactions in 35, 40 and 45% *BSA* solutions.

| *BSA* | $T_F$ [K] | $T > T_F$* | | | $T < T_F$** | |
|---|---|---|---|---|---|---|
| concentration [% w/w] | | $E_A$ [kJ/mol] | $\tau_{c0}$ [ps] | $\tau_c$ [ps] for $T$ = 293 K | $E_A$ [kJ/mol] | $\tau_{c0}$ [ps] |
| 35 | 258 | 16.1 | 0.042 | 31 | 18.4 | 0.15 |
| 40 | 263 | 14.5 | 0.11 | 42 | 19.8 | 0.076 |
| 45 | 263 | 14.3 | 0.11 | 39 | 19.6 | 0.084 |

* To fit the data the value of second moment $\langle \Delta H^2 \rangle = 30 \cdot 10^{-8}$ T$^2$ was taken; ** to fit the data the value of second moment $\langle \Delta H^2 \rangle \approx 30 \cdot 10^{-8}$ T$^2$ was calculated from the minimum of $lnT_1$ vs. *1000/T*.

tative for all measured probes. At a certain temperature we observe a discontinuity in $T_1$. This phenomenon is connected with the freezing process of the part of water at given temperature $T_F$. Moreover for the temperatures higher than $T_F$ the linear dependence of *ln* $T_1$ vs. *1000/T* is observed. It implies that the fast motion condition ($\omega\tau_c \ll 1$) is fulfilled. For this range of temperature the calculated values of activation energies (see Table II) are similar to the value of pure water: $E_A$ = 15.8 kJ/mol obtained by Hindman (1974). The values of correlation times calculated for $T$ = 293 K are longer than the $\tau_c$ of the pure water in the same temperature: 3.1 ps. The evident change of water behaviour in the concentrated samples in comparison with the pure water is visible for the temperature lower than the $T_F$ , where the dependence: $lnT_1$ vs. *1000/T* is non-linear and exhibits a distinct minimum for all samples. The value of the correlation time: $\tau_c$ = 1.6 ns from the minimum position at $T$ = 239 K was calculated. As was mentioned above in biological systems the only freezable water is *bulk* water. The spin-lattice relaxation time of ice is very short and we can't see it in our measurements (Blinc and Gränicher, 1975). Thus the activation parameters obtained in this temperature range characterise the molecular motion of *bound* (nonfreezable) water only (Szuminska *et al.*, 2000). The obtained values of $E_A$ and $\tau_{c0}$ are relatively high and suggest the serious reduction of the reorientational ability of this group of water molecules. It suggests that the processes, which influence $T_1$, may be the surface migration and anisotropic reorientation of the *bound* water similar to observed in the mammalian lenses (Bodurka *et al.*, 1996a). Therefore the measured values of spin-lattice relaxation times of *bound* water protons ($T_{1B}$) are approximately the same for all the probes.

*Spectral measurements*

The 270 MHz $^1$H spectra of different *BSA* solutions were measured. The distinct broadening of the lines while the concentration of protein in the solution is increased was observed. The numerical analysis of the spectra reveals that for the lowest concentrations (≤25%) the spectral line shapes are close to the Lorentzian profile ($L(\nu)$). For the upper limit of concentrations (≥30%) the Gaussian profile ($G(\nu)$) component gradually becomes more evident. The numerical analysis of the line shapes in this concentration range shows that the best fit is achieved for the superposition of Lorentzian and Gaussian profiles:

$$I(\nu) = L(\nu) + G(\nu) = \left[1 + \frac{4(\nu - \nu_0)^2}{(\Gamma^L)^2}\right]^{-1} + \exp\left[-\frac{(2\ln 2 \cdot (\nu - \nu_0))^2}{(\Gamma^G)^2}\right], \quad (5)$$

where $\Gamma^L$ and $\Gamma^G$ are the half-widths of Lorentzian and Gaussian profiles respectively.

To verify the slow exchange hypothesis we have estimated the values of $\tau_{ex}$ for high concentrated solutions using the fact of the Gaussian broadening of its proton spectral lines. This kind of the line broadening is connected with the local static field inhomogeneities within the probe. In the case when the exchange processes between the protons in the sample do not exist two broadened spectral lines of two groups with different chemical shifts are apparent in the spectrum. The increase of the

exchange rate between protons leads to the coalescence of these two lines to the single spectral line (Tao *et al.*, 2000) and further to its narrowing. In the case of the extremely fast exchange the spectral line should have the pure Lorentzian lineshape. The moderately slow exchange leads to the complex line shape consisting of the Lorentzian and Gaussian components. The Gaussian component is present in the spectral line as a result of the not complete time averaging of the local field inhomogeneities within the probe. Thus, it is possible to estimate the position at the spectrum where the Lorentzian profile fails and Gaussian becomes more appropriate. This position measured from the centre of the line is approximately equal to the mean exchange frequency between two proton groups in the probe ($\nu_{ex} \approx \nu - \nu_0$). To calculate the value of $\nu_{ex} = 1/\tau_{ex}$ one can transform the measured proton spectra to the co-ordinates in which the Lorentzian or Gaussian components, given by Eqn. (5), are linear respectively. Fig. 4 shows the representative example of these transformations for the 46% concentrated *BSA* solution spectral line. The estimated values of $\tau_{ex}$, in the 30%–46% concentrations range, are listed in the Table I in comparison with the measured values of spin-spin relaxation times. According to Eqn. (2) the value of the mean exchange time, $\tau_{ex}$, between the protons groups is approximately equal to the value of the mean residence time of the proton in the more mobile group. From the comparison of the $T_{2A}$, $T_{2B}$ and $\tau_{ex}$ for two most concentrated solutions one can easily see that the mean exchange time is approximately equal to the spin-spin relaxation time of *bound* water protons. Thus, the slow exchange condition ($\tau_B \geq T_{2B}$) is fulfilled (Winkler and Michel, 1985). Hence, two measured values of spin-spin relaxation times should be attributed to the *bound* and *bulk* protons groups.

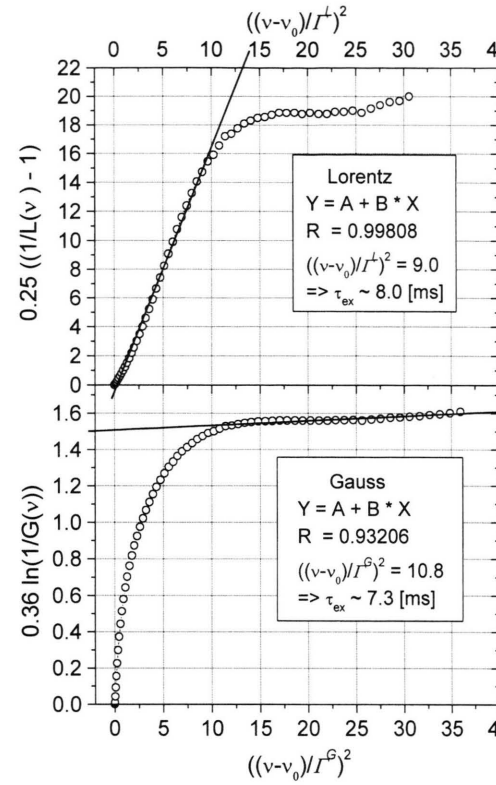


Fig. 4. The example of the line shape transformations of the 46% concentrated *BSA* solution spectral line. Upper and lower parts of Fig. 4 show the half of the 46% spectral line in the co-ordinates in which the Lorentzian ($L(\nu)$) and Gaussian ($G(\nu)$) components are linear respectively. Solid lines represent the linear fits to the transformed spectrum.

## Conclusions

Our experiments provide the evidence that the anomalous water behaviour similar like in mammalian lenses is observed in the most concentrated *BSA* solutions. Furthermore, we show that it is caused by the restrictions of *bound* water molecules mobility. In contrast to the mammalian lenses, where the water molecules ordering is present (Bodurka *et al.*, 1995), the range of the mobility restrictions depends mainly on the protein concentration in the system. In the protein solutions these restrictions are observed in relatively higher values of the protein concentration than in the lenses. We realise that the proposed hypothesis about the nonvanishing residual magnetic dipolar interactions among the protons in the *bound* water molecules is not proved in the sufficient way and further investigation is necessary. The similar measurements for the other kinds of protein solutions should be performed as well.

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Ahmad S.-B., Packer K.-J. and Ramsden J.-M. (1977), The dynamics of water in heterogeneous system. II. Nuclear magnetic relaxation of the protons and deuterons of water molecule in system with identically oriented planar interface. J. Molec. Phys. **33**, 857–874.

Allerhand A. and Gutowski S. (1965), Spin-echo NMR studies of chemical exchange. II. Closed formulas for two sites. J. Chem. Phys. **42**, 1587–1597.

Blicharska B.-O., Florkowski Z., Hennel J.-W., Held G. and Noack F. (1970), Investigation of protein hydration by proton spin relaxation time measurement. Biochim. Biophys. Acta **207**, 381–390.

Blinc A., Lahajnar G., Blinc R., Zidansek A. and Sepe A. (1990), Proton NMR study of the state of water in fibrin gels, plasma and blood clots. Magn. Res. Med. **14**, 105–122.

Blinc R. and Gränicher H. (1975), Proton dipolar spin-lattice relaxation in hexagonal ice. Z. Physik B. **22**, 211–217.

Bodurka J., Buntkowsky G., Gutsze A. and Masierak W. (1999), NMR study of role of the cross-relaxation effect in the cortex and the nucleus rabbit lens fragments. Colloids Surf. **158**, 115–119.

Bodurka J., Seitter R.-O., Kimmich R. and Gutsze A. (1997), Field-cycling nuclear magnetic resonance relaxometry of molecular dynamics at biological interfaces in eye lenses: The Lévy walk mechanism. J. Chem. Phys. **107**, 5621–5624.

Bodurka J., Buntkowsky G., Gutsze A. and Limbach H.-H. (1996a), Evidence of surface diffusion of water molecules on proteins of rabbit lens by $^1$H-NMR relaxation measurements. Z. Naturforsch. **51c**, 81–90.

Bodurka J., Buntkowsky G., Olechnowicz R., Gutsze A. and Limbach H.-H. (1996b), Investigation of water in normal and dehydrated rabbit lenses by $^1$H NMR and calorimetric measurements. Colloids Surf. **115**, 55–62.

Bodurka J., Buntkowsky G., Gutsze A. and Limbach H.-H. (1995), Evidence of anisotropic reorientation of water molecules in the cortex of the rabbit lens detected by $^1$H-NMR spectroscopy. Z. Phys. Chem. **190**, 99–109.

Cohen-Addad J.-P. (1974), Effects of the anisotropic chain motion in molten polymers: The solidlike contribution of the nonzero average dipolar coupling to NMR signals. Theoretical description. J. Chem. Phys. **60**, 2440–2453.

Cohen-Addad J.-P., Vogin R. (1974) Molecular motion anisotropy as reflected by a "pseudo-solid" nuclear spin echo: Observation of chain in molten *cis*-1,4-polybutadiene. Phys. Rev. Lett. **33**, 940–943.

Daszkiewicz O.- K., Hennel J.-W., Lubas B. and Szczepkowski T. (1963), Proton magnetic relaxation and protein hydration. Nature **200**, 1006–1007.

Edzes H.-T. and Samulski E.-T. (1978), The measurements of cross-relaxation effects in the proton NMR spin-lattice relaxation of water in biological systems: hydrated collagen and muscle. J. Magn. Reson. **31**, 207–229.

Gutsze A., Bodurka J., Olechnowicz R., Buntkowsky G. and Limbach H.-H. (1995), $^1$H-NMR and calorimetric measurements on rabbit eye lenses. Z. Naturforsch. **50c**, 410–418.

Hindman J.-C. (1974), Relaxation processes in water: viscosity, self-diffusion, and spin-lattice relaxation. A kinetic model. J. Chem. Phys. **60**, 4488–4496.

Kalk A. and Berendsen H.-J.-C. (1976), Proton magnetic relaxation and spin diffusion in proteins. J. Magn. Reson. **24**, 343–366.

Kimmich R., Klammler F., Skirda V.-D., Serebrennikova I.-A., Maklakov A.-I. and Faktullin N. (1993), Geometrical restrictions of water diffusion in aqueous protein systems. A study using NMR field-gradient techniques. Appl. Mag. Reson. **4**, 425–440.

Kimmich R., Nussep W. and Gneiting T. (1990), Molecular theory for nuclear magnetic relaxation in protein solutions and tissue: surface diffusion and free-volume analogy. Colloids Surf. **45**, 283–302.

Koenig S.-H., Brown R.-D.III and Ugolini R. (1993), A unified view of relaxation in protein solutions and tissue, including hydration and magnetisation transfer. Magn. Reson. Med. **29**, 77–83.

Lammana R. and Cannistraro C. (1989), Water proton self-diffusion and hydrogen bonding in aqueous human albumin solutions. Chem. Phys. Lett. **164**, 653–656.

Levitt M.-H., Freeman R. (1981), Compensation for pulse imperfections in NMR spin-echo experiments. J. Magn. Reson. **43**, 65–80.

Luz Z. and Meiboom S. (1963), Calculation of spin-echo decay rate in the presence of chemical exchange. J. Chem. Phys. **39**, 366–370.

Sykes B.-D., Hull W.-E. and Snyder G.-H. (1978), Experimental evidence for the role of cross-relaxation in proton nuclear magnetic resonance spin lattice relaxation time measurements in proteins. Biophys. J. **21**, 137–146.

Szuminska K., Gutsze A. and Kowalczyk A. (2000), The temperature dependence of proton spin-lattice relaxation times in highly concentrated protein solutions. Molec. Phys. Reports **29**, 153–156.

Tao T., Pan V.-H., Zhou J.-W. and Maciel G.-E. (2000), $^{13}$C NMR lineshapes of acetone adsorbed on silica. Solid State Nucl. Magn. Reson. **17**, 52–75.

Winkler H. and Michel D. (1985), Exchange processes in NMR. Adv. in Coll. and Interf. Sci. **23**, 149–177.