C. R. Physique 11 (2010) 128–135

Contents lists available at ScienceDirect

Comptes Rendus Physique

www.sciencedirect.com

Multiscale NMR and relaxation / RMN et relaxation multi-échelles

Dynamics of water in and around proteins characterized by ^{1}H-spin-lattice relaxometry

Caractérisation par relaxométrie spin-réseau des protons de la dynamique de l'eau à l'intérieur et autour des protéines

Robert G. Bryant

Chemistry Department, University of Virginia, P.O. Box 400319, Charlottesville, VA 22904-4319, USA

ARTICLE INFO

Article history:
Available online 27 July 2010

Keywords:
Water
Protein
Spin-lattice relaxation
Diffusion
Relaxation dispersion
Protein dynamics

Mots-clés :
Eau
Protéine
Relaxation spin-réseau
Diffusion
Dispersion de relaxation
Dynamique des protéines

ABSTRACT

Nuclear magnetic spin-lattice relaxation rate constants measured as a function of the magnetic field strength over wide ranges of Larmor frequency map the noise spectrum that drives spin relaxation. For water in and around protein systems, the spin relaxation reports on the average local translational mobility at the interface which is reduced by approximately factor of three from the bulk and there is anisotropy induced in the motions caused by the excluded volume created by the presence of the protein. Water also penetrates the protein and relatively few bound water sites provide a strong coupling between the protein dynamics and the water-proton-spin relaxation.

RÉSUMÉ

Les vitesses de relaxation magnétique spin-réseau mesurées en fonction de l'intensité du champ magnétique sur de grandes plages de fréquences de Larmor permettent de suivre la densité spectrale du bruit qui induit la relaxation. Pour les mouvements de l'eau à l'intérieur et autour des systèmes de protéines, la relaxation de spin mesure la dynamique de translation moyenne à l'interface protéine/eau qui est réduit d'un facteur trois par rapport à celle de la solution. Il y a de plus un effet d'anisotropie induit par les mouvements moléculaires venant du volume exclu par la présence de la protéine. L'eau pénètre la protéine également et un très petit nombre de sites d'eau liée qui engendre un fort couplage entre la dynamique de la protéine et la relaxation des protons de l'eau.

1. Introduction

Water is the ubiquitous solvent of the natural environment and is of major interest because it may directly or indirectly control critical processes. The structure and dynamics of water in the vicinity of biological molecules is important for several reasons: (1) Water as the solvent supports transport of substrates, products, and regulator molecules that modulate function to and from catalytic venues; (2) Water may be a chemical participant as a reactant or product of a reaction; (3) Water may modify the structure or dynamics, and therefore function, of the macromolecular catalysts involved in biochemical

E-mail address: rgb4g@virginia.edu.

1631-0705/$ – see front matter
doi:10.1016/j.crhy.2010.06.013

transformations. There are many approaches to characterizing water including diffraction, spectroscopic, thermodynamic, and computational methods [1–5]. The focus of this discussion will be on nuclear magnetic resonance relaxation studies of water in protein systems, but a number of conclusions are appropriate to nonbiological systems as well. Magnetic resonance experiments differ from most in that the intrinsic frequency of the measurement is low and a linear function of the magnetic-field strength used, and therefore, sensitive to long time correlations or dynamically slow events. The penalty for the low frequency of NMR spectroscopy is that the observations may yield time averaged or spatially averaged quantities rather than reports of instantaneous structures or dynamics that are spatially or structurally resolved.

A consequence of the low frequency of NMR experiments is that the spin-relaxation is not spontaneous, but must be stimulated or driven. The origin of the relaxation is coupling of the nuclear spin system to magnetic-field fluctuations at the Larmor frequency which arise from the magnetic noise created by motions of the spin-bearing molecules. Thus, the magnetic-field dependence of the spin-lattice relaxation rate constant yields the frequency dependence of the molecular dynamics that create this noise, which include intramolecular motions, intermolecular motions such as relative translation of one molecule past another, and chemical exchange or atom transfer events [6,7]. The magnetic-field dependence of the spin-lattice-relaxation-rate constant for water protons or the magnetic relaxation dispersion will be the focus of this discussion.

Although there is a long history of competing interpretative approaches in NMR relaxation [8–19] a number of points have emerged as general conclusions. The first is that although there may be variously structured water around different regions of a chemically heterogeneous structure represented by a protein, the time scale for the NMR observation windows accessible to date have provided no resolution of them; i.e., only a time average of possible structural states is reported. Except as noted below, resolution of different chemical shifts for different water molecules in a solution environment is unlikely because the frequency differences between the resonances can be of order several kHz at the highest magnetic fields available. Chemical exchange among different environments in times short compared with the reciprocal of this frequency is assured. Thus, for example, clathrate structures around hydrophobic groups such as methyl [20] will not be observable even if they are an important contribution to the thermodynamic characterization of the system. The time scale for the spectral resolution is defined by the spectral frequency differences between molecules in different structures or the chemical shift differences, but the nuclear spin-lattice relaxation shifts the relevant time scale is the reciprocal of the Larmor frequency used for the resonance experiment. The Larmor frequency, which is directly proportional to the magnetic field strength, may be varied over decades, so that the experiment may sense dynamics over a wide range of frequencies; however, the relaxation times actually measured are typically a millisecond or longer. Therefore, the observed relaxation rate constants are averaged by exchange processes that are rapid compared with these times, which includes most significant events in solution with the exception of some hydrogen chemical exchange from functions such as amides. The interpretation of the relaxation rate constants usually involves appreciation of what specific components contribute to this averaging process.

2. The rotational dispersion

An early observation about proteins in solution based on fluorescence depolarization was that the rotational correlation time for proteins was generally longer than predicted by the Stokes, Einstein, Debye model for rotational motion of a spherical particle in a viscous medium [21,22]. The early interpretation for this larger than expected size was that protein hydration, presumed to be a layer of immobilized water at the interface, increased the effective hydrodynamic radius of the protein [23]. Now that dilute solutions may be studied, the magnetic-field dependence of the relaxation dispersion profile for protein solutions reports rotational correlation times in excellent agreement with the fluorescence depolarization results [24]. However, the magnitude of the low field relaxation rate constants are completely inconsistent with a large number of immobilized water molecules coordinated to the protein with a rotational correlation time that is essentially the same as the protein. This point will be addressed further below. The slower than expected rotational motion of the protein in water is now largely attributed to the shape and roughness of the protein interface [25].

The second point that comes from relaxation dispersion studies in solution is that there is generally a coupling between the rotational motion of the protein and the solvent as shown in Fig. 1. There has been some debate about the origin of this coupling that has included the effects of bound solvent molecules or labile protons mixing with the bulk water, and loose hydrodynamic coupling between the water and the protein [26]. The magnetic relaxation dispersion associated with the rotational motion of proteins is observed in a variety of solutes, and a relaxation coupling between the protein and the solvent protons is retained when the protein rotational motion is stopped by chemical cross-linking reactions. There is now consensus that the dominant cause for the solvent proton relaxation dispersion that reports the rotational mobility of proteins is the binding and exchange of only a few solvent molecules [8,12]. The chemical exchange of labile protons from protein functional groups such as amines or accessible amides also contributes to the rotational coupling between water and the protein; this contribution is dependent on the pH of the solution. The mixing of relaxation rate constants in the relatively dilute solution case is given by

$$\frac{1}{T_1} = \frac{P_f}{T_{1f}} + \sum_i \frac{P_{bi}}{T_{1bi} + \tau_i} \tag{1}$$

P_f and T_{1f} are the probability and relaxation time in the bulk water environment and P_b and T_{1b} are the probability and relaxation times in the bound environments, i. In the case that the exchange lifetimes are short compared with the

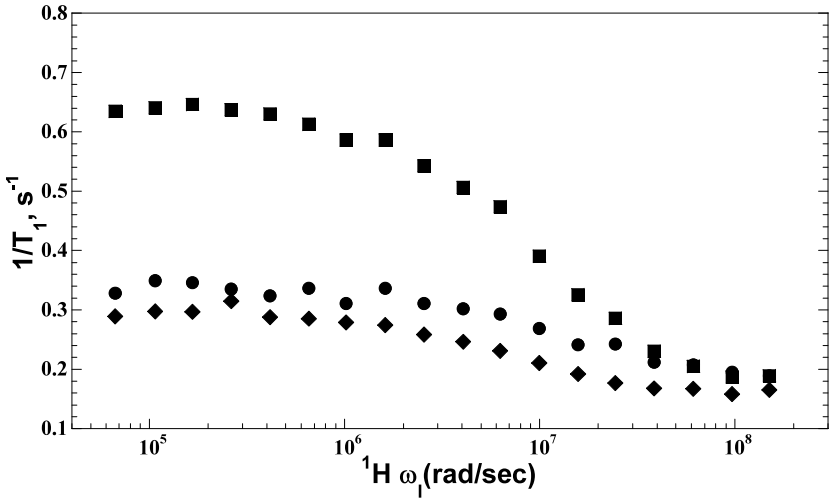

Fig. 1. The ^{1}H-spin-lattice relaxation rate constant plotted as a function of the magnetic field strength represented as the proton radial frequency for a 300 μM bovine serum albumin solution at a nominal pH of 7 in a mixed solvent of 200 mM acetone (■), 200 mM dimethylsulfoxide (◆), and water (●) diluted with D_2O to approximately match the proton concentrations with the other solvents.

relaxation times in the bound environment, which is expected for most of the bound water sites, the observed relaxation rate constant becomes the weighted sum of the relaxation times in each environment. The proton exchange contributions may be reduced by the effects of the mean lifetime, τ_i, in the bound environment, and in some cases this may be so long that a site makes no significant contribution to the relaxation rate constant.

In solution, the proton intramolecular contribution, or the deuteron and oxygen-17 relaxation are relatively easily modeled because the correlation time for the relaxation is the rotational correlation time for the intramolecular vectors between the hydrogen atoms of the water molecule or along the electric field gradient directions for the deuteron and oxygen nuclei. The structure and magnetic parameters for water molecules are well known. For a water molecule bound to the protein, the magnetic coupling between the two protons may be directly computed and the relaxation rate constant calculated as well if the rotational correlation time is known. In the fast exchange limit of Eq. (1), knowledge of the bound T_1 values based on the rotational relaxation rate constant provides the probability that the molecules are bound to the protein, therefore, the number of bound water molecules. Although the relaxation mechanisms are different, deuterium, and oxygen measurements of these numbers are in excellent agreement with the proton based measurements [9,12,15,24]. All three of these methods share an ambiguity associated with the possibility of some degree of high frequency local motion of the water. What is actually obtained from the relaxation analyses is the product of the number of bound water molecules and the square of the order parameter, which introduces a numerical factor between 0 and 1 thought for several reasons to be close to 1 for most bound molecules. Nevertheless, these approaches provide the best method presently available for characterizing the number of long-lived bound water molecules associated with a protein. In spite of this modest numerical uncertainty, the data show clearly that there are not hundreds of bound water molecules associated with the protein in the interfacial region of the solution. It is apparent in Fig. 1 that the approach is not limited to characterizing bound water molecules; any resolved resonance may be characterized this way provided there is an observable relaxation dispersion.

3. The high frequency dispersion

Instrumental advances have provided the opportunity to measure the relaxation dispersion of protein systems to high magnetic-field strengths above the rotational dispersion shown in Fig. 1. The magnetic-field dependence is weak and is represented by a power law in the frequency with a small exponent, or a logarithmic dependence on the Larmor frequency. We note that if oxygen is present, there is a substantial contribution to proton relaxation from the electron–nuclear coupling modulated by translational diffusion of the water. Oxygen effects in aqueous solutions are interesting in their own right [27], but beyond the scope of the present discussion. In the present discussion, we presume that the solution has been purged of oxygen.

The difficulty with a weak magnetic-field dependence is that there are not sharp features that permit ready distinction between alternative explanations for the observation. Two different approaches are envisioned for the magnetic-field dependence in a protein solution above the rotational inflection; they are not mutually exclusive. The weak magnetic-field dependence could result from a broad distribution of rotational and translational correlation times for the water molecules in the interfacial region. Alternatively, the geometry of the interface may impart a reorientational bias to the water molecules so that the diffusive motion appears to be dimensionally restricted in the interface. 2-dimensional confinement is well known to provide a logarithmic magnetic-field dependence for the relaxation rate constant of a liquid diffusing in a layered space [28]. Employing this model to the high frequency dispersion data of three proteins provided a self consistent set of

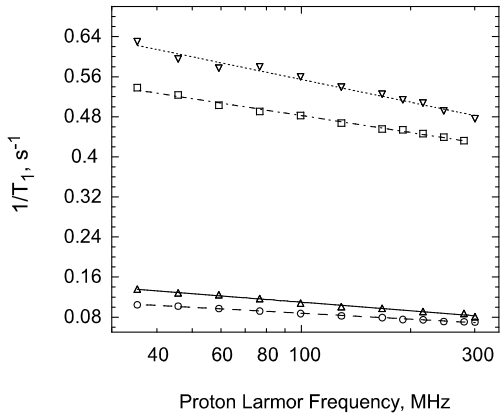

Fig. 2. The ^{1}H-spin-lattice relaxation rate constant plotted as a function of the magnetic field strength represented as the proton Larmor frequency for 1.5 mM bovine serum albumin (triangles), and 6.8 mM lysozyme aqueous solutions at neutral pH (circle and square) in H_2O (top) and D_2O (bottom) where the residual HOD protons were observed.

diffusion constants for water that is in excellent agreement with other approaches [29]. The translational correlation time for water moving at the protein surface was found to be 30 ps, about three times slower than in pure water. Of course, this is an aggregate characterization and is somewhat dominated by the slower contributions to the total measurement. Nevertheless, the result from the high frequency diamagnetic solutions is in agreement with completely different measurements made in paramagnetic solutions where the electron–nuclear dipolar coupling modulated by the relative translational diffusion dominates the relaxation process [30].

An important feature of the logarithmic dependence is that the lifetime in the interfacial region for at least some of the water molecules must be long compared with the reciprocal of the Larmor frequency, which for the proteins studied implies that some molecules may diffuse in the interfacial region for times of order ns. This possibility is supported by several molecular dynamics simulations; however, there are many molecules with shorter lifetimes as well. A feature of the relaxation dispersion is that the rate constant may be dominated by the slow correlation times in the problem, and therefore, care is required when trying to characterize the average molecule and its motion. It is interesting that a model calculation in which the reencounter probability is examined as a function of the thickness of the interfacial layer and diameter of the macromolecule suggest that the effects of the interfacial diffusive barrier may be present even for relatively small globular macromolecules [29].

A different approach to such data is to assume that there is a distribution of rotational and translational correlation times in the interfacial region. The rotational motion is usually modeled by a Lorentzian relaxation equation, while the translational motion is more complex. In either case, a broad distribution is required to provide the dependence shown in Fig. 2. That some distribution of dynamics is associated with the interface is intrinsically reasonable for molecules like proteins because there is a chemical heterogeneity associated with a protein interface created by different amino acid side chains. In addition, this heterogeneity carries a nonuniform charge distribution that is also a function of the solution pH. Finally, the protein surface is far from smooth, which as noted above is one source of the discrepancy between the apparent radius of the macromolecule and the rotational reorientational time. At present, it is difficult to make a clear distinction between these contributions or eliminate one. We note that the surface translational model provides a relatively simple origin for the observed magnetic-field dependence that gives self consistent values for the diffusion constant of water at the interface.

4. Dynamics and spin dynamics in immobilized protein systems

There is no reason to expect that the water molecule dynamics in the interfacial region of a protein should change when the rotational motion of the protein is stopped by intermolecular coupling of some kind such as chemical cross-linking. There is no evidence from the nuclear spin relaxation that the surface dynamics are different; i.e., the translational mobility remains rapid and this fact is responsible in part for the ease of observing nuclear magnetic resonance images in tissues that are molecularly crowded. However, the nuclear spin relaxation in nonrotating protein systems is qualitatively and quantitatively different from that in solutions. The magnetic-field dependence of the spin-lattice relaxation becomes a power law in the Larmor frequency [16,31–33] and this change from Lorentzian shapes for the solution occurs at constant composition and therefore reflect only the spin dynamics in the protein that change when the rotational mobility is quenched as shown in Fig. 3.

Several factors change when the protein stops rotating that are critical for spin relaxation [16,33]. First, the proton–proton dipolar coupling between protein protons is not rotationally averaged so that the protein-spin system behaves magnetically like a solid. Spin–spin communication among the protein protons is rapid, the transverse relaxation time is short, of order 10 μs, and consequently, the linewidth of the protein-proton spectrum is about 35 kHz. The linewidth far exceeds the proton

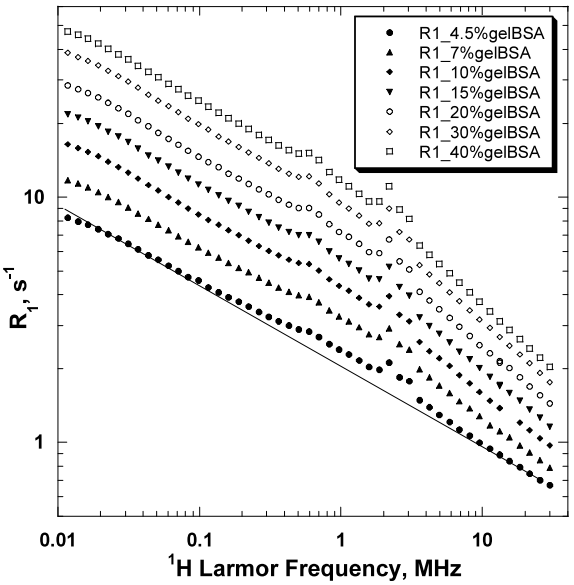

Fig. 3. The ^{1}H-spin-lattice relaxation rate constant plotted as a function of the magnetic field strength represented as the proton Larmor frequency for bovine serum albumin gels crosslinked with glutaraldehyde for protein concentrations from 4.5% (bottom) to 40% by weight (top). The straight line shown at the bottom is shown for reference. The peaks approximately 0.7, 2.4 and 2.8 MHz are caused by proton coupling to the ^{14}N of the peptide and arise when the proton transitions match the nitrogen transitions to provide another relaxation pathway via the efficient nitrogen relaxation.

chemical shift range. Thus, essentially all protein protons contribute to a single resonance line that is homogeneous; i.e., one may not apply a selective irradiation in one portion of the line without simultaneously affecting the whole line shape. Further, the effects of a selective irradiation that saturates the protein spectrum are carried by magnetization transfer from the protein spins to the water spins. An irradiation of the protein line far from the water resonance saturates the protein line but the water line as well, which is the basis for what is called z-spectroscopy [34]. This magnetic coupling is the basis for the magnetization transfer imaging methods in clinical radiology. Because a liquid magnetic resonance line such as the water, or that of a small solute with a narrow linewidth, responds to the irradiation of the solid, the spectrum of the solid may be probed by observing the liquid line [35,36], which is generally less technically demanding than direct detection and may avoid difficult dynamic range problems in the detection of the solid. One consequence of the magnetic coupling between the protein and the water spins is that the water spins may report the magnetic-field dependence of the immobilized protein spins. Therefore, simple interpretations of the water-spin-lattice relaxation in such heterogeneous environments based on isotropic rotational models are inappropriate. In short, the spin-lattice relaxation rates do not simply reflect the water dynamics in a rotationally immobilized protein system.

To understand the magnetic relaxation in the immobilized protein case, it is convenient to begin with a dry protein and examine the behavior with increasing water content. The dry protein spin-lattice-relaxation-rate constants change with the magnetic-field strength according to a power law that is related to the dynamics of the polypeptide backbone [32]. Fluctuations in the backbone cause modulations of the proton–proton dipolar couplings that then drive spin relaxation. A self consistent model for this process has been presented that is related to spin-phonon models but because the protein is irregularly packed and locally heterogeneous, aspects of its structure are fractal and the effective lattice is not infinite. Thus, the model is a spin-fracton model that involves a direct rather than a Raman process where there are energy conserving transitions in the lattice to compensate for the transitions in the nuclear spin system [17,18]. This model predicts that the relaxation rate constant is a linear function of the temperature, which is observed over 150 K temperature range. It also provides the power law exponent in terms of two parameters that characterize the dimensionality for the distribution of mass in space, d_f, and the power law exponent that characterizes the vibrational distribution of states, d_s, which is smaller than 2; i.e., smaller than the Debye limit. It is not the purpose of this discussion to examine the details of this model; we only note that it summarizes simply a considerable body of data and predicts correctly both the temperature and magnetic-field dependence of the protein-proton-spin-lattice relaxation [19,37–40].

The addition of water to the protein system hydrates strong water binding sites first which drives some structural rearrangement of the protein and adds protons to the relaxation problem that may be dynamically distinct from the protein protons. It is important to note that in the case where the protein is hydrated with D_2O, the protein-proton relaxation remains a power law in the frequency over the whole range of water contents up to 90% water by weight [41]. Therefore, the underlying protein dynamics are preserved with the addition of water, which may alter the high frequency local motions of the protein and modify the detailed structure as the strains from electrostatic interactions in the dry protein are relieved by the addition of mobile electric dipoles to the environment. However, when H_2O is used, the protein-spin system may communicate with the water-proton-spin system as summarized in Eq. (2). The effect of the coupled relaxation is that the

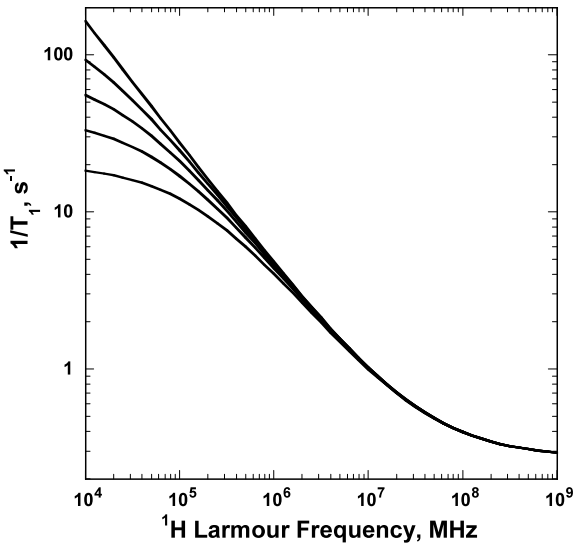

Fig. 4. Water proton nuclear spin-lattice relaxation rate constants as a function of the magnetic field strength represented as the proton Larmor frequency computed based on Eq. (2) where R_P is assumed to be a power law with a relaxation rate at 0.01 MHz of 1500 s^{-1}, F is held at 0.1, and the magnetization transfer rate constant R_{WP} takes values of 20, 40, 80, 200, and 10^5 s^{-1} and R_W is 3 s^{-1}.

approach of the longitudinal magnetization to equilibrium is biexponential [42,43]. In most cases, the slow time constant is observed which is given

$$\frac{1}{T_{1s}} = \frac{1}{2}\left\{\frac{1}{T_{1W}} + \frac{1}{T_{1P}} + \frac{1}{T_{WP}}\left(1+\frac{1}{F}\right) - \left[\left(\frac{1}{T_{1P}} - \frac{1}{T_{1W}} - \frac{1}{T_{WP}}\left(1-\frac{1}{F}\right)\right)^2 + \frac{4}{FT_{WP}^2}\right]^{\frac{1}{2}}\right\} \tag{2}$$

where F is the ratio of the number of solid protons to the number of water protons, $\frac{1}{T_{1P}}$ is the protein-proton-relaxation rate given by a power law in the Larmor frequency [17]; $\frac{1}{T_{WP}}$ is the pseudo first order rate constant for magnetization transfer between protein and water-proton populations, and $\frac{1}{T_{1W}}$ is the bulk water-relaxation rate constant. At all magnetic-field strengths, the observable relaxation constants scale with the relative size of the two proton populations [8].

One effect of this communication or coupled relaxation is to reduce the measurable relaxation rate constants for the protein protons which act as the relaxation sink for all the protons. The water proton relaxation rates scale as $F/(1+F)$ where F is the ratio of the number of protein protons to the number of protein protons. Even if the underlying dipolar coupling between the protein protons and the water protons is independent of water content, a second consequence of the water addition is that as the water pool grows, the effective magnitude of the cross-relaxation-rate constant between the protein pool and the water pool decreases, which is essentially a consequence of detailed balance. This change may result in a low field plateau in the water spin relaxation rate when the water spin-lattice relaxation is limited by the effective magnetization transfer to the protein spins that serve as the relaxation sink as summarized in Fig. 4.

In addition to these effects of the size of the two coupled proton populations, the relaxation rate constant, $1/T_{WP}$, between the water and the protein spins may be a function of the local water dynamics, temperature, and frequency. In the limit that water is strongly held in the long-lived binding sites, the water spins are indistinguishable from the protein spins and are magnetically an integral part of the solid-spin system. The coupling between these water spins and the protein is efficient, and the rate constant is of order $1/T_2$ for the immobilized spins or approximately 10^5 s^{-1}. Because water in these rapidly relaxed but rare bound-water environments mix by magnetic and chemical exchange with other water on the protein, the effective rate constant for the magnetization transfer decreases as indicated above. If there is local high frequency wiggling of the water molecules in a strongly bound environment, the effective magnitude of the dipolar coupling between the water spins and the protein spins will be reduced. The common example of this effect is the rapidly rotating methyl group and the scaling factor that characterizes the reduction is usually represented as the square of an order parameter characterizing the geometric constraints of the high frequency exploration. The high frequency or fast motion characterized by a correlation τ_f may also add a dispersion to the relaxation profile when $\omega\tau_f \sim 1$ may or may not be apparent depending on the magnitudes of other contributions to the relaxation-rate constants.

A second contribution to the relaxation dispersion may arise when the residence time of the water molecule in the tightly bound environments is short. One consequence of this short lifetime is that the effective cross-relaxation rate constant for communication between the water and protein spins may decrease. As a result, the exchange plateau will move to higher frequency. Halle has built a model for the relaxation dispersion of the water protons in immobilized protein systems that ignores the effects of the magnetic-field dependence of the protein protons, but examines the effects of the water exchange rates on the dipolar relaxation that is largely dominated by the intramolecular water proton-water–proton coupling,

but also includes nearest neighbor protein protons [44]. The assumption of the model is that the spin–spin communication within the protein is inefficient, which is opposite to the model discussed above. This assumption makes the model a liquid-like model that appears to be inconsistent with the failure of hole-burning experiments in such protein systems. Nevertheless, the model makes an important point that the exchange events may limit the size of the dipolar coupling and the exchange event may contribute to the dispersion. Other effects of the solid aside, the exchange of the water that breaks the rigid-limit dipolar coupling would cause a dispersion in the relaxation at a frequency of the order of the first-order-exchange-rate constant. If the dipolar coupling to the protein spins involves no transfer of the protein magnetic-field dependence to the water protons, then the dispersion from a particular site has a Lorentzian magnetic-field dependence. The power-law-magnetic-field dependence is then thought to result from a broad distribution of exchange times that produce a sum of Lorentzian dispersions characterized by correlation times spanning a number of decades. Although neglect of relaxation coupling over the whole range of magnetic-field strengths seems inappropriate in light of experiments that demonstrate it, the limitations imposed by the magnetic and chemical exchange rates on the shape of the relaxation dispersion profile are important and may add to the creation of the low magnetic-field plateau as noted above.

For a water molecule in a rare strongly bound site, the proton spectrum is indistinguishable from the protein-proton spectrum and although there are contributions to the water-proton dipolar linewidth from the other proton on the same oxygen atom as well as all the protein protons, there is no particular value in distinguishing intra from intermolecular contributions to the total coupling. In this context, we note that the spin-fracton theory for protein-proton relaxation include specifically the assumption that the protein spins are well coupled to each other as in other solids. Halle has recently reported an important experiment involving heavily deuterated protein [45]. In the case that the protein is fully deuterated, there is no protein-proton-spin bath, and no coupling possible of water protons to protein protons except rare exchangeable NH or OH proton sites. Thus, the relaxation dispersion in such a system is dominated by the water-proton intramolecular coupling that may be modulated by local motions in the bound environments and by chemical exchange events. Since the rare protein protons are largely decoupled from each other, the spin-fracton theory for the protein-proton spin-lattice relaxation rate constant that depends on efficient proton–proton communication is not relevant for this case, nor is it tested by such an experiment.

A feature that appears when the water proton relaxation dispersion experiment is conducted over a wide range of magnetic-field strengths is the appearance of an approximately Lorentzian contribution that is absent at the same concentrations when D_2O is used [41]. This contribution has been ascribed to local motion of bound water molecules in part because similar contributions have been observed in hydrated systems at low temperature where exchange events are improbable. However, for the room temperature cases, it is not possible to directly rule out the contributions from chemical exchange events on at least some of the bound water sites making this contribution [44,45]. In any case, the reciprocal of the rate constants for this process are on the order of tens of ns. Contributions from this source are supported in part by the recent experiments of Halle and coworkers. Whether the dynamics involve local motion of the bound water molecules or exchange, because water molecules carry electric dipole moments, water motions in this range may contribute the effective dielectric characterization of the protein environment and modulate the kinetic or thermodynamic barriers for reorientation of protein or substrate components.

5. Conclusion

Magnetic relaxation dispersion measurements on protein systems in solution and immobilized environments provide a picture of water dynamics that are slightly modified at the protein interface. The motion at the interface may be anisotropic and when considered in aggregate, the average dynamics appear to be slowed by approximately a factor of 3 relative to pure water and ice-like water does not dominate the dynamics of the interface down to the time scale of order 10 ps. Proteins differ from most inorganic substrates in that they are penetrable to some extent and often carry several long-lived, perhaps buried, water molecule sites that are central to coupling the water-spin relaxation to the rotational and spin-relaxation properties of the protein. In the solution case, these sites provide a coupling mechanism for the water spins to the rotational motion of the protein. In the rotationally immobilized case, these sites provide a primary coupling between the protein and the water spins that carries the magnetization transfer between the water and protein proton populations. The chemical exchange of the water molecules from the rare protein sites to the surrounding water environments is critical in carrying the cross relaxation between the protein spins and the water spins as well as a source of water-proton relaxation in its own right.

References

[1] H. Frauenfelder, E. Gratton, Protein dynamics and hydration, Methods Enzymol. 127 (1986) 207–216.
[2] H. Frauenfelder, B. McMahon, Dynamics and function of proteins: The search for general concepts, Proc. Natl. Acad. Sci. USA 95 (1998) 4795–4797.
[3] H. Frauenfelder, B.H. McMahon, R.H. Austin, K. Chu, J.T. Groves, The role of structure, energy landscape, dynamics and allostery in the enzymatic function of myoglobin, Proc. Natl. Acad. Sci. USA 98 (2001) 2370–2374.
[4] C. Mattos, Protein–water interactions in a dynamic world, Trends Biochem. Sci. 27 (2002) 203–208.
[5] S.K. Pal, A.H. Zewail, Dynamics of water in biological recognition, Chem. Rev. 104 (2004) 2099–2123.
[6] A. Abragam, The Principles of Nuclear Magnetism, The Clarendon Press, Oxford, 1961 (chapter viii).
[7] M. Goldman, Quantum Description of High-Resolution NMR in Liquids, Clarendon Press, Oxford, 1988.
[8] R.G. Bryant, The dynamics of water–protein interactions, Annu. Rev. Biophys. Biomol. Struct. 25 (1996) 29–53.

[9] B. Halle, V.P. Denisov, K. Venu, Multinuclear relaxation dispersion studies of protein hydration, in: L.J.B.N.R. Krishna (Ed.), Biological Magnetic Resonance, Kluwer Academic/Plenum, New York, 1999, pp. 419–484.
[10] B. Halle, M. Davidovic, Biomolecular hydration: From water dynamics to hydrodynamics, Proc. Natl. Acad. Sci. USA 100 (2003) 12135–12140.
[11] S.H. Koenig, R.D.D. Brown, R. Ugolini, A unified view of relaxation in protein solutions and tissue, including hydration and magnetization transfer, Magn. Reson. Med. 29 (1993) 77–83.
[12] V.P. Denisov, B. Halle, Protein hydration dynamics in aqueous solution, Faraday Discuss. 103 (1996) 227–244.
[13] V.P. Denisov, J. Peters, H.D. Horlein, B. Halle, Using buried water molecules to explore the energy landscape of proteins, Nat. Struct. Biol. 3 (1996) 505–509.
[14] B. Halle, V.P. Denisov, A new view of water dynamics in immobilized proteins, Biophys. J. 69 (1995) 242–249.
[15] B. Halle, Protein hydration dynamics in solution: A critical survey, Philos. Trans. Royal Soc. London B 359 (2004) 1207–1224.
[16] R.G. Bryant, D.A. Mendelson, C.C. Lester, The magnetic field dependence of proton spin relaxation in tissues, Magn. Reson. Med. 21 (1991) 117–126.
[17] J.P. Korb, R.G. Bryant, The physical basis for the magnetic field dependence of proton spin-lattice relaxation rates in proteins, J. Chem. Phys. 115 (2001) 10964–10974.
[18] J.P. Korb, R.G. Bryant, Magnetic field dependence of proton spin-lattice relaxation times, Magn. Reson. Med. 48 (2002) 21–26.
[19] J.P. Korb, R.G. Bryant, Noise and functional protein dynamics, Biophys. J. 89 (2005) 2685–2692.
[20] P.J. Rossky, M. Karplus, Solvation. A molecular dynamics study of a dipeptide in water, J. Am. Chem. Soc. 101 (1979) 1913–1937.
[21] D.R. Bauer, J.I. Brauman, R. Pecora, Molecular reorientation in liquids. Experimental test of hydrodynamic models, J. Am. Chem. Soc. 96 (1974) 6840–6843.
[22] D. Kivelson, P.A. Madden, Light scattering studies of molecular liquids, Annu. Rev. Phys. Chem. 31 (1980) 523–558.
[23] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum Press, New York, London, 1983, p. 143.
[24] S. Kiihne, R.G. Bryant, Protein–bound water molecule counting by resolution of ^{1}H spin-lattice relaxation mechanisms, Biophys. J. 78 (2000) 2163–2169.
[25] A. Van-Quynh, S. Willson, R.G. Bryant, Protein reorientation and bound water molecules measured by ^{1}H magnetic spin-lattice relaxation, Biophys. J. 84 (2003) 558–563.
[26] R.G. Bryant, NMR relaxation studies of solute–solvent interactions, Ann. Rev. Phys. Chem. 29 (1978) 167–188.
[27] C.-L. Teng, B. Hinderliter, R.G. Bryant, Oxygen accessibility to ribonuclease a: Quantitative interpretation of nuclear spin relaxation induced by a freely diffusing paramagnet, J. Phys. Chem. A 110 (2006) 580–588.
[28] J.P. Korb, M. Winterhalter, H.M. McConnell, Theory of spin relaxation by translational diffusion in two-dimensional systems, J. Chem. Phys. 80 (1984) 1059–1068.
[29] D. Grebenkov, Y.A. Goddard, G. Diakova, J.P. Korb, R.G. Bryant, Dimensionality of diffusive exploration at the protein interface in solution, J. Phys. Chem. B 113 (2009) 13347–13356.
[30] C.F. Polnaszek, R.G. Bryant, Nitroxide radical induced solvent proton relaxation: Measurement of localized translational diffusion, J. Chem. Phys. 81 (1984) 4038–4045.
[31] R. Kimmich, F. Winter, W. Nusser, K.H. Spohn, Interactions and fluctuations deduced from proton field-cycling relaxation spectroscopy of polypeptides, DNA, muscles, and algae, J. Magn. Reson. 68 (1986) 263–282.
[32] R. Kimmich, F. Winter, Double-diffusive fluctuations and the $\nu^{3/4}$-law of proton spin-lattice relaxation in biopolymers, Prog. Colloid Polym. Sci. 71 (1985) 66–70.
[33] C.C. Lester, R.G. Bryant, Water-proton nuclear magnetic relaxation in heterogeneous systems: Hydrated lysozyme results, Magn. Reson. Med. 22 (1991) 143–153.
[34] J. Grad, R.G. Bryant, Nuclear magnetic cross-relaxation spectroscopy, J. Magn. Reson. 90 (1990) 1–8.
[35] D.P. Hinton, R.G. Bryant, Measurement of protein preferential solvation by z-spectroscopy, J. Phys. Chem. A 98 (1994) 7939–7941.
[36] D.P. Hinton, R.G. Bryant, 1h magnetic cross-relaxation between multiple solvent components and rotationally immobilized protein, Magn. Reson. Med. 35 (1996) 497–505.
[37] J.P. Korb, R.G. Bryant, Magnetic field dependence of proton spin-lattice relaxation of confined proteins, C. R. Physique 5 (2004) 349–357.
[38] Y. Goddard, J.-P. Korb, R.G. Bryant, The magnetic field and temperature dependences of proton spin-lattice relaxation in proteins, J. Chem. Phys. 126 (2007) 175105/175101–175105/175105.
[39] Y. Goddard, J.-P. Korb, R.G. Bryant, Nuclear magnetic relaxation dispersion study of the dynamics in solid homopolypeptides, Biopolymers 86 (2007) 148–154.
[40] J.-P. Korb, G. Diakova, Y. Goddard, R.G. Bryant, Relaxation of protons by radicals in rotationally immobilized proteins, J. Magn. Reson. 186 (2007) 176–181.
[41] G. Diakova, A. Goddard Yanina, J.P. Korb, R.G. Bryant, Water and backbone dynamics in a hydrated protein, Biophys. J. 98 (2010) 138–146.
[42] W.M. Shirley, R.G. Bryant, Proton nuclear spin relaxation and molecular dynamics in the lysozyme–water system, J. Am. Chem. Soc. 104 (1982) 2910–2918.
[43] H.T. Edzes, E.T. Samulski, Cross relaxation and spin diffusion in the proton NMR of hydrated collagen, Nature 265 (1977) 521–523.
[44] B. Halle, Molecular theory of field-dependent proton spin-lattice relaxation in tissue, Magn. Reson. Med. 56 (2006) 60–72.
[45] E.P. Sunde, B. Halle, Slow internal protein dynamics from water ^{1}H magnetic relaxation dispersion, J. Am. Chem. Soc. 131 (2009) 18214–18215.