Magnetic Field Dependence of Proton Spin-Lattice Relaxation Times

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The magnetic field dependence of the water-proton spin-lattice relaxation rate ($1/T_1$) in tissues results from magnetic coupling to the protons of the rotationally immobilized components of the tissue. As a consequence, the magnetic field dependence of the water-proton ($1/T_1$) is a scaled report of the field dependence of the ($1/T_1$) rate of the solid components of the tissue. The proton spin-lattice relaxation rate may be represented generally as a power law: $1/T_1\omega = A\omega^{-b}$, where b is usually found to be in the range of 0.5–0.8. We have shown that this power law may arise naturally from localized structural fluctuations along the backbone in biopolymers that modulate the proton dipole-dipole couplings. The protons in a protein form a spin communication network described by a fractal dimension that is less than the Euclidean dimension. The model proposed accounts quantitatively for the proton spin-lattice relaxation rates measured in immobilized protein systems at different water contents, and provides a fundamental basis for understanding the parametric dependence of proton spin-lattice relaxation rates in dynamically heterogeneous systems, such as tissues. Magn Reson Med 48:21–26, 2002. © 2002 Wiley-Liss, Inc.

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The value of the water-proton spin-lattice relaxation time, T_1, is often an important determinant of magnetic image contrast. Therefore, understanding the magnetic field dependence of T_1 is central to a fundamental understanding of the factors that control the magnetic resonance (MR) image appearance as well as the information content. It is well known that the magnetic field dependence of the water-proton T_1 is dominated by magnetic coupling to the protons of the solid components of the tissue. As summarized in Fig. 1, the observed magnetic field dependence of $1/T_1$ was shown to be a scaled representation of the magnetic relaxation dispersion of the protons in the rotationally immobilized spin components of the tissue (1,2). The magnetic coupling is carried by chemical-exchange events that include a whole water molecule exchange between specific binding sites, and proton exchange with protein functions such as amines, amides, and alcohols. Because proton-exchange is generally much slower than H_2O exchange, the coupling is usually dominated by water molecule exchange events. In the bound environment of these unique water molecules, the magnetization transfer rate is limited, essentially by the value of T_2, which is of the order of 10 μs in the bound and immobilized environment (2–4). However, the origin of the magnetic field dependence of $1/T_1$ in the rotationally immobilized proton spin system of a protein, for example, is not quantitatively understood. Kimmich and coworkers (13) have suggested that the field dependence derives from the backbone fluctuations. Our recent theoretical work (5) provides a quantitative explanation for the magnetic field dependence of $1/T_1$ for the solid component spins, which, in turn, provides an explanation for the magnetic field dependence of the water-proton $1/T_1$ in tissues. Although a variety of macromolecular assemblies may contribute to the T_1 in tissues, including lipids, saccharides, nucleotides, and proteins, we have focused on protein examples because they are likely to be the dominant factor determining the magnetic relaxation rates in most tissues. However, the concepts are general and applicable to a variety of dynamically heterogeneous materials in which the relatively immobilized components are chain molecules.

Magnetic spin-lattice relaxation is stimulated in that it requires coupling of the nuclear spins to the magnetic noise at the Larmor frequency in the system. This coupling provides the spin system with a pathway to establish thermal equilibrium with the other degrees of freedom in the sample, collectively referred to as the lattice. The magnetic noise derives from the complete spectrum of molecular dynamic events, including vibration or libration, rotation, translation, and conformational interconversion. In a molecular solid, there is often a considerable distribution of local motions present, so some components of the solid relax rapidly compared with others. For protons, spin-spin coupling constants are large, and protons communicate efficiently by spin diffusion in solids or in systems in which rotational motion is quenched. In this case the relatively immobile spins relax indirectly through the more mobile ones for which the relaxation rate is high. These effects of spin diffusion are well documented in solid proteins, in which the proton spin-lattice relaxation rate in the dry case at high field strengths is determined by spin diffusion to rapidly rotating methyl groups associated with amino acids, such as alanine (6,7). In hydrated systems near laboratory temperatures, water molecule motions add additional sites of rapid motion that also behave as relaxation sinks (8). As a consequence, the temperature dependence of protein-proton T_1 values at 1.5 T is dominated by the high-frequency motions of these relaxation sinks; however, the magnetic relaxation dispersion profile from Larmor frequencies of 0.01 MHz to several MHz reports the effects of motions at frequencies well below the rapid methyl or water molecule rotations.

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The spin-lattice relaxation rate for protein protons in solid samples, as well as in tissues, is described by a power law of the form

$$\frac{1}{T_1(\omega)} = A\omega^{-b} + C \qquad [1]$$

where A is a constant, b is usually between 0.5 and 0.8, and C is a constant that represents the relaxation rate plateau at high field. Representative data are shown in Fig. 2. In a dry protein, b is generally about 0.75, but approaches 0.5 when hydrated (1,4,9–15). The power law implies that this field dependence is significant: a factor of 2 reduction in the field produces a 30% change in $1/T_1$. In the limit of a uniform lattice, it has been known for a long time that spin diffusion to a relaxation sink, such as a paramagnetic impurity, results in a power law like that in Eq. [1] with $b = 0.5$ (16). In this case, spin diffusion to the relaxation sink must be slow compared with the relaxation rate at the sink; however, for biomolecules and proteins in particular, just the opposite is true; i.e. spin diffusion rates are large compared to relaxation rates at methyl or water sites. Thus, in diamagnetic solid biomolecules, the power law may not derive from the effects of slow diffusive exploration of the spin-space to relax at a rare relaxation sink.

In this work we outline the essential features of our previously described (5,17) ^{1}H spin relaxation model and apply it to purified protein systems as a model for tissue systems. Of course, the tissue system is more complex and must represent a superposition of the effects of different proteins and other macromolecular assemblies. However, the essential features and physical content follow from the protein case. Finally, we analyze the low-frequency plateau in terms of a simple but detailed model for the primary cross-relaxation pathway.

MATERIALS AND METHODS

Description of the Model

Nusser and Kimmich (13) presented qualitative arguments a number of years ago suggesting that the power law for the magnetic field dependence of the proton spin-lattice relaxation in protein systems was tied to the backbone motions. We have shown that this result derives naturally from the fractal character of the proton distributions in folded proteins. Stapleton et al. (18) and Allen et al. (19), in considering electron spin relaxation in heme proteins, showed that the carbon atom positions in proteins form fractal systems. We have computed the fractal dimension, d_f, for the C_α positions in hen egg white lysozyme based on the x-ray coordinates, and find that $d_f(C_\alpha) = 1.76 \pm 0.1$. This fractal dimension is small, which means that the connections between chain segments are weak and that structural fluctuations will be localized. Furthermore, unlike hard lattice solids, such as ionic crystals, a backbone fluctuation does not propagate like a wave; rather, we assume stochastic localized structural excursions. Calculations of the proton fractal dimension for lysozyme, α-chymotrypsin, and ribonuclease A from the crystal structure yield values of 2.50 ± 0.05. The protein proton spin system thus forms a percolation network at or above the percolation threshold. The proton connectivity is loose and not uniform in space, but is sufficient to ensure efficient spin diffusion.

A theoretical development presented elsewhere (5) quantitatively models the effects of localized quasi-vibrational fluctuations on the proton dipole-dipole couplings that cause nuclear spin-lattice relaxation. We have considered explicitly the time fluctuations induced by localized longitudinal and librational motions of the protein backbone. In fact, such a localization is responsible for the typical power law found for the magnetic field dependence of the spin lattice relaxation rate. A crucial aspect of

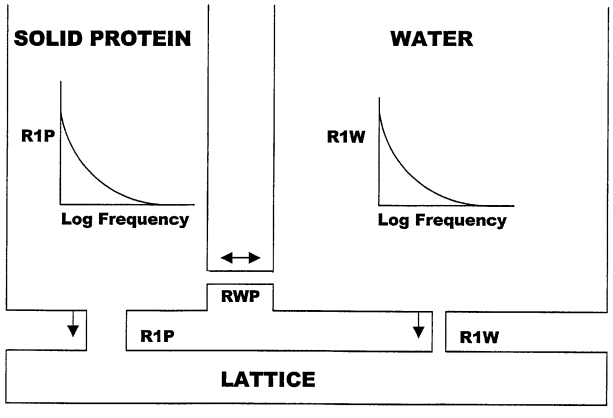

FIG. 1. Schematic representation of the proton spin-lattice relaxation in dynamically heterogeneous systems in which the magnetic field dependence of the solid component is transferred to the water protons by several chemical-exchange processes—predominantly the exchange of a few water molecules.

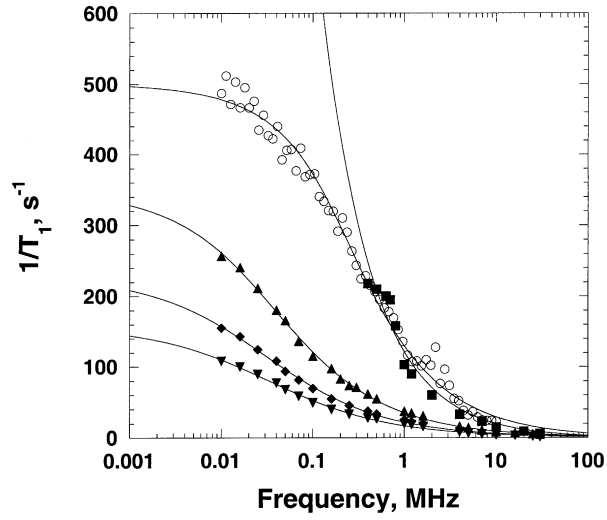

FIG. 2. The spin-lattice relaxation rates measured as a function of magnetic field strength reported as the proton Larmor frequency at 291 K for a lysozyme dry and hydrated to various levels with H_2O: ■ dry lysozyme; ○ 20% water by weight; ▲ 35% water by weight; ◆ 50% water by weight; ▼ 55% water by weight (1). The solid lines are best fits to the data computed using Eqs. [2] and [4], where only b and R_{WP} were permitted to vary. The parameters are summarized in Table 1. The apparent noise in the 20% data in the range of 0.7–3 MHz is caused by the well-known effects of the proton Zeeman levels crossing the ^{14}N levels dominated by the nuclear electric quadrupole coupling.

the theoretical development is that the effectiveness of the nuclear spin-coupling to the distribution of motions in the essentially solid matrix of the protein is made several orders of magnitude more efficient than expected for spin-phonon coupling in a 3D crystalline system because of the effectively reduced dimensionality of the chain structure of the protein. The folded protein chain is stiff along the chain, but the between-chain couplings are soft and the effective dimensionality for the dominant motions is thus reduced; i.e., the critical motions that cause proton spin relaxation involve the small displacements in the primary structure. Proteins share this characteristic with all linear polymers, or weakly cross-linked polymers. Although proteins may have cross-links (e.g., lysozyme has two disulfide bonds), these are relatively rare, so the predominant disturbances are along the chain. Following the development of Abragam (16) for spin-phonon coupling as a relaxation mechanism, modified to account for the nonuniform proton distribution and the localization of structural fluctuations, we find (5):

$$\frac{1}{T_1} = \frac{9\pi\beta d_s}{10}\left(\frac{k_B T}{\hbar}\right)\left\{\left(\frac{3}{4}\right)\left(1+\frac{1}{2^b}\right)\left(\frac{\hbar\omega_{dip}}{E^0_{v\|}}\right)^2\left(\frac{\hbar\omega_0}{2E^0_{v\|}}\right)^{-b} + \left(\frac{1}{6}\right)\left(\frac{7}{2}+\frac{1}{2^b}\right)\left(\frac{\hbar\omega_{dip}}{E^0_{v\perp}}\right)^2\left(\frac{\hbar\omega_0}{2E^0_{v\perp}}\right)^{-b}\right\} \quad [2]$$

$$b = 3 - \frac{2d_s}{d_f} - d_s \quad [3]$$

where d_s is the spectral dimension that is related to the localized character of the vibrations and enters from the characterization of the density of vibrational states. However, the value is found by experiment and numerical modeling to be 4/3 for all dimensions of interest to MRI, so we may treat this factor as a constant (20–23). $E^0_{v\|}$ and $E^0_{v\perp}$ are the energies of the highest vibrational modes relevant to the system parallel and perpendicular to the direction of the chain, ω_{dip} is the magnitude of the proton-proton-dipole coupling estimated as 11.3 kHz for protons separated by 2.2 Å with $\beta \sim 3$ based on the proton linewidth, ω_0 is the proton Larmor frequency, and d_f is the fractal dimensionality characterizing the spatial proton distribution in the protein. The vibrational energy, E_v, enters because one must consider all vibrational modes up to the highest-frequency mode. Although the distribution spans many decades, the highest-frequency vibration enters as the limit of the integration over the distribution, which we approximate as the value of the amide II frequency 1560 cm^{-1} associated with the polypeptide backbone of the protein (24). This choice provides quantitative agreement with experiment. Because d_s is fixed at 4/3 (22), b is determined by the fractal dimension of the protons, d_f, which directly reflects the uniformity of the distribution of protons in space.

Comparison With Experiment

According to Eq. [3] in the limit that $d_f = 3$, b becomes 0.78, which is very close to the values reported for dry proteins that are more compact than hydrated ones (25). The value of d_f computed from the crystal structures of lysozyme and ribonuclease A are 2.50 ± 0.05, which corresponds to $b = 0.61$. This change corresponds to an increase in the nonuniformity of the proton distribution when the protein is hydrated from the lyophilized state. Tissue relaxation dispersion profiles are generally characterized by values of b close to 0.5; however, the analysis usually applied does not directly incorporate the effects of cross-relaxation in the characterization of the power-law exponent.

Figure 2 shows proton spin-lattice relaxation rate dispersion profiles for lysozyme samples ranging from 55% water by weight to dry (5,17). Several features are clear. The dry protein is characterized by the first term of Eq. [2], as discussed previously (5), and is a power law in the proton Larmor frequency with $b = 0.78$. However, data on hydrated samples show a low-field plateau that is most obvious for the 20% sample (the data for which were collected on a field cycling instrument with 50 times faster response time than the other data, which permitted exploration of the low-field range). The low-field plateau is a direct consequence of the relaxation coupling between the water proton and the rotationally immobilized protons in the protein, as summarized schematically in Fig. 1. In both imaging and field cycling experiments, only the slow component of the nonexponential decay is generally detected, and the apparent rate constant, R_{slow} is given by

$$R_{slow} = \frac{1}{2}\left[R_W + R_P + R_{WP}\left(1+\frac{1}{F}\right) - \left[\left(R_P - R_W - R_{WP}\left(1-\frac{1}{F}\right)\right)^2 + \frac{4R^2_{WP}}{F}\right]^{0.5}\right] \quad [4]$$

where R_W and R_P are the spin-lattice relaxation rate constants for the water and protein protons, R_{WP} is the cross-relaxation rate constant between the water and the protein, and F is the ratio of the protein-proton population to the water-proton population at equilibrium (M^∞_P/M^∞_W) (1). R_W is independent of the magnetic field strength over the range studied here because the water motions are very rapid, even when in contact with the protein surface (2,26). We assume that R_{WP} is not a function of magnetic field strength because the magnetization transfer rate for a bound and rotationally immobilized proton is limited by the T_2 of the solid phase, which is approximately 10 μs for protein systems. As is further developed below, R_{WP} may be written in more physically explicit terms; however, for the moment we will treat it as an adjustable parameter.

R_P contains the magnetic field dependence of the proton relaxation and is given by Eq. [2], with all the parameters except d_f fixed as described above. With R_W set at 1 s^{-1}, and F determined from the composition, the data shown in Fig. 2 were then fit to Eq. [4] by adjusting d_f and R_{WP} yielding the solid lines and the values of d_f and R_{WP} summarized in Table 1.

For the dry sample, the first term of Eq. [2] fits the data well with $b = 0.78$, which corresponds to $d_f = 3.0$, i.e., the same as the Euclidean dimension. This result implies that the distribution of protons in space is essentially uniform, and that the protein structure in the lyophilized solid is more uniform than that in the hydrated crystal, for which

Table 1
Spin-Lattice Relaxation Parameters

% Water[a]	F	b	R_{wp}, s^{-1}	R[b]	d_f[c]	k_{ex}, s^{-1}
Dry	–	0.78	–	0.977	3.00	–
20	2.38	0.68	500	0.995	2.71	1.0×10^5
35	1.11	0.614	350	0.999	2.53	1.6×10^5
50	0.596	0.605	230	0.998	2.51	1.8×10^5
55	0.487	0.597	160	0.998	2.49	1.8×10^5

[a]Composition by percent weight. Other parameters that enter via Eq. [2] are fixed as indicated in the text. R_W was fixed at 1.0 s^{-1} in all cases. The values of k_{ex} were computed using Eq. [7].
[b]Pearson's correlation coefficient.
[c]$d_f = -2d_s/(b + d_s - 3)$.

we deduce a value of $d_f = 2.5$ from the hydrogen atom positions.

The hydrated protein samples are all fit well by Eq. [4] using Eq. [2] for the value of R_P. In all cases, the relaxation rates are reduced by the effects of cross relaxation compared with that for the dry protein. The data obtained on the 20% water sample clearly show a low-field plateau caused by the limitations of a magnetization transfer rate that is less than the relaxation rate of the immobilized protein protons. The value of d_f deduced from b is 2.71, which is different from both the dry and fully hydrated values. At 20% water, the protein-charged groups are almost completely hydrated, but increasing hydration continues to modify the protein, as reflected by changes in the partial molar heat capacity up to a hydration level approaching 40% (27). The samples with higher water contents between 35% and 55% display similar behavior, with very similar values of b (and therefore $d_f \sim 2.5$), and an effective magnetization transfer rate that decreases with increasing water content. Over the range of water contents between 35% and 55% the protein is nearly fully hydrated and the details of the folded structure are not expected to change significantly, which is reflected in a nearly constant value of d_f. The water-content dependence of R_{WP} may be understood in terms of a more specific model.

There are several contributions to R_{WP}: 1) direct dipole-dipole coupling between diffusing water spins and the spins of the rotationally immobilized protein; 2) chemical exchange of labile protons between water proton sites and protein functions such as amides, amines, and hydroxyl groups; and 3) exchange of whole water molecules between the water pool and protein-binding sites that have long-lived water molecules. As discussed previously (2), the contribution to the water proton-protein proton coupling modulated by translational diffusion is very small because the effective diffusion coefficient of the water at the macromolecule surface is not very different from that in the bulk. As a result the correlation time for the coupling is short and the relaxation efficiency low. The two chemical-exchange mechanisms may both provide strong coupling between the macromolecule and the water pool. Both pathways involve water protons, but contribution 2 involves only proton exchange, while contribution 3 involves whole water molecule exchange. Both of these contributions may be modeled by an equation of the form

$$R_{WP} = \sum_{i=1}^{N} \frac{P_o}{T_{2solid} + \tau_{ex,i}} \qquad [5]$$

where the sum runs over all N labile proton sites on the macromolecule (including water sites), P_o is the probability that a proton occupies the ith site on the protein, T_{2solid} is the transverse relaxation rate for the protons in the solid or bound phase, and $\tau_{ex,i}$ is the mean residence time for the proton or water molecule at the ith site. The fundamental limitation of the magnetization transfer at each bound environment is T_{2solid}, which is approximately 10–12 μs for the proteins studied thus far (2,28,29). The exchange lifetime for each site may vary considerably between different sites. However, proton lifetimes on protein ionizable groups, which are strongly pH dependent, are generally long compared with water molecule lifetimes, which implies that the contributions at neutral or acid pH values from proton exchange will be limited substantially by the effects of the exchange times $\tau_{ex,i}$ in the denominator of Eq. [5]. Consequently, we will focus our discussion on the whole water molecule mechanism, while recognizing that proton exchange may contribute to some extent as well.

The probability for a labile protein-proton site exchanging with water may be written as

$$P_o = \frac{18n}{2dM_p^o} = \frac{18n(1-p)}{2pM_p^o} = \frac{nF}{N_H} \qquad [6]$$

where n is the number of labile protons at the site (2 for a water molecule), d is the ratio of the mass of water in the sample to the mass of protein or macromolecule, M_P^o is the molecular weight of the protein, p is the weight percent water, F is the ratio of the number of protein protons to the number of water protons, and N_H is the number of protons in the protein (973 for lysozyme). Although progress is being made in characterizing exchange lifetimes of individual water molecules at protein-binding sites, we generally do not know the lifetime at each site, even if we may know the number of sites (30–33). Nevertheless, we may write Eq. [5] as

$$R_{WP} = P_o \sum_{i,sites}^{N} \frac{1}{T_{2solid} + \tau_{ex,i}} = P_o k_{ex} \qquad [7]$$

where k_{ex} is the sum over the effective exchange contributions from each site. The experimental value of k_{ex} includes the sum of all exchange contributions between the protein and the water, including any labile proton sites that are distinct from water molecule binding sites. In the case that all exchange lifetimes are very short, k_{ex} becomes

N/T_{2solid}, where N is the number of long-lived sites on the protein. In the case that the chemical exchange lifetimes limit the denominator for some sites, the sum will be less than N/T_{2solid}. The lifetimes for the long-lived water molecules bound to the protein are estimated to range from about 0.05 μs to tens of μs (34,35). The proton lifetimes on labile sites such as amines, alcohols, and amides may have a wide distribution of lifetimes that depend significantly on pH. At low and neutral pH, almost all are slow compared with labile water exchange. It is well known that different proteins have different numbers of long-lived water molecules, which average of order several water molecules per 10 kD of molecular weight for proteins (34). For example, serum albumin, a 68 kD protein, has 25 ± 3 long-lived water molecules if local order parameters are neglected. Thus, the effective magnetization transfer rate will increase with the number of long-lived water molecule binding sites, which is a function of protein structure and conformation.

The concentration dependence of the magnetization transfer rate is contained in P_o. Rearranging Eq. [7] to solve for k_{ex}, we find the values of k_{ex} listed in Table 1 for the corresponding value of R_{WP}. The other entries in Table 1 are F, the ratio of the protein spin population to the water spin population that enters the cross-relaxation equation; b, the experimental value of the power law exponent found from the field dependence of the $1/T_1$; R_{wp}, the cross relaxation rate between the water and the protein spins deduced from the fit to Eq. [4], with Eqs. [2] and [3] substituted for R_P; R, Pearson's correlation coefficient associated with the fit of Eq. [4] to the data in each case; and d_f, the fractal dimension deduced from Eq. [2]. The average value of k_{ex} taken over all water contents is 1.5×10^5 s^{-1}. The modest scatter in the derived values of k_{ex} may result from small differences in protein conformation that may alter the exchange rates of water, or small differences in pH and contributions from labile proton positions. We note in this context that the values of k_{ex} from 35% to 55% samples have consistent values in the range of $1.7 \pm 0.2 \times 10^5$ s^{-1} over this water content range where the protein is nearly fully hydrated. Taking the value of T_{2solid} as 12 μs, and assuming that all water molecule lifetimes are short compared with 12 μs, we may estimate the number of long-lived water molecules that contribute to the value of R_{WP}, assuming that no other labile protein protons make a significant contribution. In this case, the value of k_{ex} corresponds to 1.8 long-lived water molecule sites on lysozyme. This value is consistent with the small number of labile long-lived water molecules deduced by other approaches for lysozyme (36).

CONCLUSIONS

The theory for proton dipole-dipole coupling modulated by localized fluctuations in chain molecules that are rotationally immobilized combined with the effects of water-macromolecule cross-relaxation account quantitatively for the observed power law for water-proton spin-lattice relaxation in model protein systems. The primary parameters are the fractal dimensionality of the proton distribution in the structure of the macromolecular matrix and the effective spin exchange rate constant between the macromolecule protons and the water protons, which is simply related to the number of long-lived water molecule sites as well as to the number of labile protons and their exchange lifetimes. This two-parameter model is easily generalized to more complex systems, such as tissues, because all rotationally immobilized molecules that contribute are linear polymers with relatively few cross-links. While this theoretical treatment provides a foundation for understanding the magnetic field dependence of proton spin-lattice relaxation in more complex systems, including tissues, the distribution of molecular species and local concentrations present in these systems makes interpretation of effective values of d_f and k_{ex} problematic at a molecular level. Nevertheless, this development provides a conceptual framework for understanding the magnetic spin-lattice relaxation dispersion in terms of a simple characterization of the macromolecular structure, d_f, and simple kinetic parameters summarized collectively in k_{ex}.

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