Journal of Magnetic Resonance 199 (2009) 68–74

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Water molecule contributions to proton spin–lattice relaxation in rotationally immobilized proteins

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ARTICLE INFO

Article history:
Received 7 January 2009
Revised 1 April 2009
Available online 8 April 2009

Keyword:
Spin–lattice relaxation, Magnetic relaxation dispersion
Water dynamics
Solid proteins
Field dependence

ABSTRACT

Spin–lattice relaxation rates of protein and water protons in dry and hydrated immobilized bovine serum albumin were measured in the range of ^{1}H Larmor frequency from 10 kHz to 30 MHz at temperatures from 154 to 302 K. The water proton spin–lattice relaxation reports on that of protein protons, which causes the characteristic power law dependence on the magnetic field strength. Isotope substitution of deuterium for hydrogen in water and studies at different temperatures expose three classes of water molecule dynamics that contribute to the spin–lattice relaxation dispersion profile. At 185 K, a water ^{1}H relaxation contribution derives from reorientation of protein-bound molecules that are dynamically uncoupled from the protein backbone and is characterized by a Lorentzian function. Bound-water-molecule motions that can be dynamically uncoupled or coupled to the protein fluctuations make dominant contributions at higher temperatures as well. Surface water translational diffusion that is magnetically two-dimensional makes relaxation contributions at frequencies above 10 MHz. It is shown using isotope substitution that the exponent of the power law of the water signal in hydrated immobilized protein systems is the same as that for protons in lyophilized proteins over four orders of magnitude in the Larmor frequency, which implies that changes in the protein structure associated with hydration do not affect the ^{1}H spin relaxation.

1. Introduction

The motions of water in or on biological macromolecules are of fundamental importance because the dynamics modulate intra- and inter-molecular energetics as well as macromolecular structure [1–4]. Although enormous progress has been made in characterizing the dynamics of water–protein systems, understanding remains incomplete. Nuclear magnetic relaxation dispersion (MRD), the measurement of nuclear spin–lattice relaxation-rate constants as a function of magnetic field strength, offers valuable information on molecular dynamics and structure. Nuclear spin relaxation is not spontaneous, but derives from coupling of the nuclear spins to the magnetic noise in the system, which in turn arises from molecular motion. The magnetic field dependence of the spin–lattice relaxation rate, i.e., the ^{1}H MRD profile, then provides a quantitative statistical characterization of the molecular dynamics that drive the spin relaxation; usually this is a map of the frequency dependence for the intra- and inter-molecular magnetic dipolar couplings.

Previous MRD studies of dry proteins have shown that the relaxation is described by a power law in the Larmor frequency, $\frac{1}{T_1} = A\omega^{-b}$, where A and b are constants [5–10]. The physical origin of the power law has been related to a spin–fracton relaxation mechanism [6,11–15]. The essential dynamical picture behind this relaxation mechanism is similar to those employed in vibrational network models for protein dynamics [16–18]. The propagation of structural fluctuations in the protein which modulate ^{1}H dipolar couplings that drive spin relaxation are characterized by a reduced dimensionality because of the limited or non-uniform connectivity in the folded protein structure [6,7,19]. The exponent, b, in the power law is related by the relaxation theory to a spectral dimension, d_s, which characterizes the vibrational density of states and the dimensionality of the disturbance propagation, and a fractal dimension, d_f, which describes the distribution of mass in space [6]. For dry proteins $b = 0.76 \pm 0.04$ [5,6,10,15,20,21]. In the rotationally immobilized systems, spin–spin couplings are efficient and a common spin temperature is established rapidly. As a consequence, motions that relax one group efficiently relax the whole ^{1}H spin population, which is observed as a single broad resonance line. Recent MRD studies of dry proteins and polypeptides over wide temperature ranges revealed the nature of the side-chain contributions to the ^{1}H spin relaxation [21,22]. At high and low frequencies, the field dependence is a power law because the main-chain fluctuations also modulate the side-chain couplings. A displacement of the high and low frequency power laws is

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doi:10.1016/j.jmr.2009.04.001

caused by the side-chain motions, which create a transition when the side-chain frequency approximates the proton Larmor frequency. The effects of the side-chain dynamics move into the experimental frequency range only at low temperatures using currently convenient magnetic fields [21,22]. The present experiments focus on relaxation contributions of protein-bound water dynamics, which in some cases look like a side-chain contribution in that the water motions may be coupled to the backbone dynamics. For other water molecules, the local motions are uncoupled from the backbone dynamics, which makes a relaxation contribution that may be distinguished from the coupled case based on the shape of the MRD profile.

Fig. 1 illustrates magnetic relaxation dispersion profiles for dry and hydrated bovine serum albumin at 302 K. The hydrated protein system is a valuable model of a more complex counterpart such as a tissue where the physical and chemical diversity of the components make detailed analysis problematic. A distinct and critical feature of the water ^{1}H relaxation dispersion profile of the heterogeneous water–protein system (Fig. 1) is that, analogous to the dry protein system, it is described by a power law in magnetic field strength or ^{1}H Larmor frequency. The efficient magnetization transfer or cross relaxation between protein and water–proton spins is responsible for this effect and has been widely studied [2,8,23–26]. The cross relaxation affects the response of both the water- and protein-spin populations. The usual model presumes that there are relatively few water molecules that are bound to the protein for times of hundreds of nanoseconds to several microseconds and is supported by solution phase MRD measurements that count the number of such molecules [27]. These unique molecules affect the relaxation rate of the whole water population through protein–water-proton and water–water-proton dipolar interactions coupled with proton and water molecule exchange from bound to bulk environments. In earlier work, the changes in the MRD profile on hydration were attributed to changes in the power law exponent given by $b = 3 - 2\frac{d_S}{d_f} - d_S$ [6,15]. The change in b was ascribed to structural changes in the protein upon hydration that affected the special distribution of protons, and therefore, d_f [6]. In this paper we reexamine this intriguing issue and show that the water content dependence of the power law exponent is not supported by more complete data sets that span a larger range of temperature and frequency. Further, studies at low temperature reveal bound-water-molecule motions that are independent of the protein-backbone fluctuations and characterized by a Lorentzian relaxation dispersion profile.

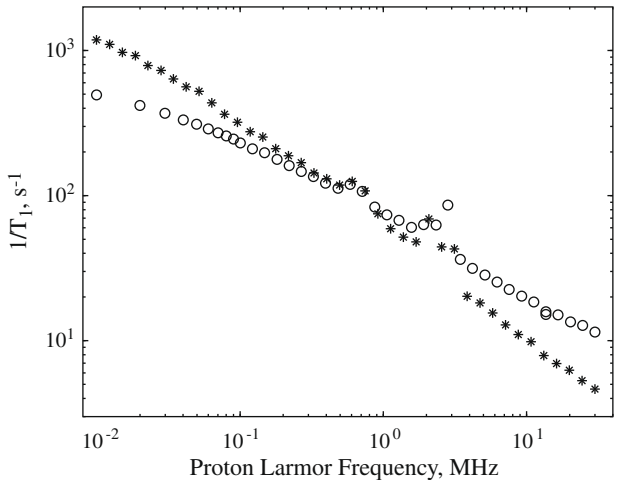

Fig. 1. The proton spin–lattice relaxation-rate constants as a function of magnetic field strength plotted as the proton Larmor frequency for dry (stars) and hydrated to 0.32 g H_2O/g protein (open circles) bovine serum albumin at 302 K. The peaks in the relaxation profiles of all samples between 0.5 and 5 MHz are due to ^{14}N–^{1}H level crossing [45].

2. Experimental

Bovine serum albumin (BSA) obtained from Sigma Chemical Company (St. Louis, MO) was dialyzed against at least five changes of deionized water. The protein was lyophilized using a mechanical vacuum at 298 K. Solvated samples were prepared by adding the desired mass of solvent, such as deionized water or deuterium oxide (99.9 atom % D, Cambridge Isotope Laboratories, Inc., Andover, MA) to a known mass of protein. Hydrated protein samples were allowed to equilibrate for at least 3 days at 310 K. The amount of moisture in hydrated BSA samples was additionally checked by a Karl Fischer titrator (Aquatest 8, Photovolt Instruments, Inc., Indianapolis, IN). The hydrated protein samples used in this study were prepared to contain 0.32 g water per 1.0 g of protein. It has been shown that as the level of hydration of small globular proteins increases above 0.38 g water/g protein, the protein can be considered fully hydrated in a sense that further addition of water does not change its spectroscopic or thermodynamic properties as compared to fully hydrated (1.0 g water/0.1 g protein) protein gels [3,4,28–30]. Since, to first approximation, the number of water molecules in direct contact with the protein at any given time is proportional to the surface area of the protein, which correlates with molecular weight, the larger proteins are believed to be fully hydrated at slightly lower water levels [31].

For BSA samples prepared with D_2O, 1.0 g of BSA was initially dissolved in 20 mL of D_2O and stirred at 325 K for 4 h, then transferred to a Centricon filter (Millipore; 30,000 MW cut-off) and concentrated to 5 mL in the centrifuge. The concentrated solution was diluted again to 20 mL with D_2O as the procedure was repeated 4 times to minimize the number of exchangeable protons remaining on the protein. Finally, the protein was lyophilized at 337.8 K using a drying pistol with refluxing methanol and a mechanical vacuum.

The nuclear magnetic resonance data were recorded using an FFC-2000 fast field cycling NMR spectrometer (Stelar s.r.l., Mede, Italy). The Stelar spectrometer provides temporal control of the magnetic field; in the present experiments the field-switching time used was 3 ms. Proton spins were polarized at 30 MHz and free induction decays were recorded following a single (6.7 μs) 90° excitation pulse applied at 15.8 MHz [32]. The relaxation fields were varied between ^{1}H Larmor frequency 0.01 and 30 MHz. The spectrometer dead time was 11 μs. The NMR signal was averaged (at least 8 scans) for at most 32 linearly spaced time sets, each of which was adjusted at every relaxation field to optimize the sampling of the decay/recovery curves. Within experimental error, all the decay/recovery curves of longitudinal magnetization were exponential. Temperature was varied from 154 K to 302 K using a Stelar VTC90 variable temperature controller, which was calibrated using an external thermocouple inserted into a surrogate sample at the resonance position in the probe. Based on repeated calibrations, the temperature in all NMR experiments was controlled to within 0.5 K. Samples were allowed to equilibrate for at least 20 min at each temperature before data acquisition.

3. Results and discussion

The proton transverse magnetization decay of dry protein can be described well by a single Gaussian with a decay time on the order of 10 μs, but for hydrated protein powders the transverse magnetization decay is multi-component [33]. The rapid transverse decay is characteristic of solid protein. The slow transverse decay includes contributions from water- and protein-side-chain protons