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Decay of Dipolar Order in Diamagnetic and Paramagnetic Proteins and Protein Gels

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Magnetic relaxation in solids may be complicated by the creation and loss of dipolar order at finite rates. In tissues the molecular and spin dynamics may be significantly different because of the relatively high concentration of water. We have applied a modified Jeneer–Broekaert pulse sequence to measure dipolar relaxation rates in both dry and hydrated protein systems that may serve as magnetic models for tissue. In lyophilized and dry serum albumin, the dipolar relaxation time, T_{1D} is on the order of 1 ms and is consistent with earlier reports. When hydrated by deuterium oxide, the dipolar relaxation times measured were on the order of tens of microseconds. When paramagnetic centers are included in the protein, the Jeneer–Broekaert echo decay times became the order of the decay time for transverse magnetization, i.e., the order of 10 μs or less. In the hydrated or paramagnetic systems, the dipolar relaxation times are too short to require inclusion in the quantitative analysis of magnetization transfer experiments.

Key Words: T_{1D}**; dipolar relaxation; MTC; magnetization transfer;** *Z* **spectroscopy.**

INTRODUCTION

Magnetization transfer pulse sequences have provided new opportunities for changing the information available from magnetic images by superimposing the magnetic response of tissue components on the observable water proton magnetization (*1–3*). This information transfer is possible because of magnetic coupling between the solvent protons and the solid component protons. Most models developed to understand the effects of the relaxation coupling have focused on Zeeman and transient transverse magnetizations at the level of the coupled Bloch equations (*4–7*). While these approaches provide an apparently accurate description of most systems studied, concern remains that some preparation pulse schemes may create dipolar order in the solid component spin system that could change the observed response significantly (*8, 9*). We study proteins because proteins are generally capable of forming highly organized compact structures that could provide the structural and dynamic platform for the creation of dipolar order in the ^{1}H spin system. There have been several reports of the relaxation time for loss of dipolar order in proteins; however, these measurements have been made using dry lyophilized systems that may not represent accurately the local molecular dynamics in a wet tissue system (*10–12*). The general expectation has been that hydration will facilitate additional internal molecular motion that will decrease the magnitude of T_{1D} (*13*). The measurements reported here support this expectation and the assumption that dipolar order effects may be neglected in fully hydrated systems.

EXPERIMENTAL

Lyophilized protein powders were prepared from aqueous solutions containing bovine serum albumin (Sigma, 98%) which had been dialyzed to remove adventitious salts. Gel samples were made by cross-linking the protein with cold aqueous 25% glutaraldehyde at 273 K in an ice bath. Typically an 18% protein solution in 1 mL of water and 0.14 mL of D_2O were mixed with 0.5 mL of 25% glutaraldehyde (Sigma Grade II) at ice temperature. Paramagnetic gels were made by reacting a threefold molar excess of diethylenetriaminepentaacetic acid dianhydride (Aldrich Chemical Company) with a dilute serum albuin solution over ice. A typical reaction volume was 200 mL to prevent protein cross-linking by the dianhydride; the pH was kept above 7 with Hepes buffer. The resulting protein solution was concentrated and dialyzed against three changes of water to remove hydrolysis products and excess reagent. The protein was lyophilized and redissolved in water and stoichiometric amounts of metal were added volumetrically from concentrated stock solutions. Manganese sulfate and gadolinium chloride were obtained from Aldrich Chemical Company. The paramagnetic proteins were cross-linked thermally at 80°C for 20 min. These samples were lyophilized and the proton spectrum of the product showed no residual narrow water resonance. For measurements in the presence of water added as D_2O, the protein systems were subjected to three cycles consisting of dissolution in D_2O, storage at 60°C for 20 min, and lyophilization to remove labile protons. The resulting deuterated and cross-linked protein was then hydrated with D_2O to a level of 35%.

The NMR experiments were conducted at resonance frequencies of 500 and 60 MHz on a Varian Unity Plus spec-

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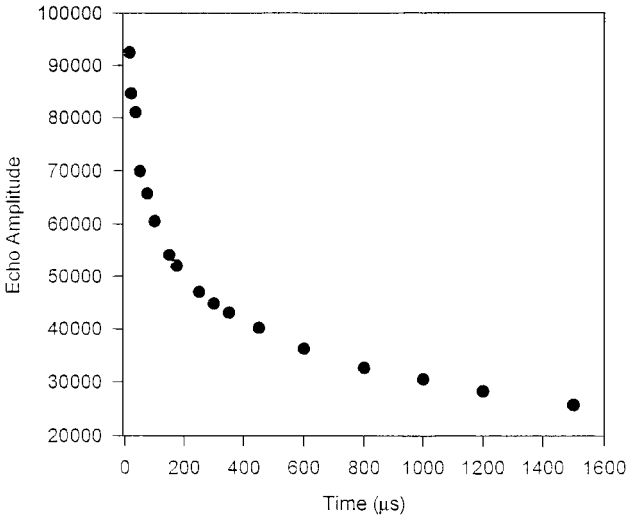

FIG. 1. Decay of the ^{1}H echo created by a modified Jeener–Broekaert pulse sequence at 295 K applied to a dry lyophilized bovine serum albumin sample at a resonance field of 1.4 T or a proton Larmor frequency of 60 MHz.

trometer and a homemade spectrometer, respectively. The 60-MHz spectrometer employed a Varian EM-360 permanent magnet and a Tecmag Libra data system. The transmitter used a PTS-160 frequency synthesizer operating into Vari-L RF gates and an ENI LPI-10 RF amplifier driving a transmission line probe built in this laboratory. The receiver consisted of a Miteq preamplifier operating into an Avantek amplifier cascade that drove a simple quadrature detector utilizing Merrimac doubly balanced mixers. The drive to the LPI-10 amplifier was attenuated to achieve 90° and 45° pulses of 2.8 and 1.4 μs, respectively. The Jeneer–Broekaert dipolar spin-echo sequence as modified by Yang and Schleich was used to minimize direct contributions from Zeeman magnetization and is summarized as $\{(90_x\text{–}\tau_1\text{–}45_y\text{–}t\text{–}45_y\text{–}\tau_2\text{–acq}(+y)) + 90_{-x}\text{–}\tau_1\text{–}45_y\text{–}t\text{–}45_y\text{–}\tau_2\text{–acq}(-y)\}$ (*12*). The values of $-\tau_1$ and $-\tau_2$ were 10 μs at both magnetic field strengths. The values of the 90° and 45° pulses used at 500 MHz were 9.2 and 4.6 μs, respectively.

RESULTS AND DISCUSSION

Figure 1 summarizes representative results for the decay of the dipolar echo signal obtained with the modified Jeneer–Broekaert pulse sequence. The dry serum albumin decay is clearly not a single exponential, but the long-time component of approximately 400 μs is in reasonable agreement with values reported for other proteins near laboratory temperature (*10–12*). The frequency domain spectrum obtained from the Fourier transform of the second half of the dipolar echo is shown in Fig. 2. The line is broad and has essentially a Gaussian shape usually observed for lyophilized proteins. The nonexponential decay of the dipolar echo may result from differences in the local motions within the protein that may be expected because local structures are distributed among helicies, sheets, and loop regions, which may have considerably different motions. The nonexponential decay may also result from the effects of magnetization transfer within the interacting spin systems and has been demonstrated to be important, particularly in heteronuclear cases in engineering polymers (*14, 15*). This nonexponential character of the decay will only be more pronounced in the case of a tissue where there is a distribution of rotationally immobilized macromolecules. However, even in this simple model case, the dipolar relaxation times are very short compared with the spin–lattice relaxation times, which are typically on the order of 0.1 to 1 s depending on the condition of the sample, the temperature, and the magnetic field strength (*16, 17*).

The changes in relaxation induced by the addition of paramagnetic centers is important because of potential applications of magnetic contrast agents that may be targeted to the rotationally immobilized components of a tissue. We use stoichiometric quantities of manganese and gadolinium ions bound to serum albumin. Representative dipolar relaxation data are shown in Fig. 3 and summarized in Table 1. The decay of the dipolar echo in all paramagnetic cases is very rapid and is on the order of the transverse relaxation time. Within our ability to measure it, the decay is exponential and substitution of deuterium for exchangeable protons on the protein makes no difference in the observed decay. The proton lineshape is unchanged from the reference diamagnetic example and we observe essentially the same results at 60 and 500 MHz for the decay of the dipolar echo amplitude. The presence of the paramagnetic centers in the protein may induce significant shifts in resonance frequency that may compromise the effectiveness of the pulse sequence for creating dipolar order. Nevertheless, to the extent that we may characterize the decay of the dipolar echo over the limited range of the observation window, the relaxation is exponential and very rapid.

The decay of dipolar order in hydrated environments is key to quantitative understanding of magnetization transfer experiments *in vivo*. The experimental problem in a hydrated sample

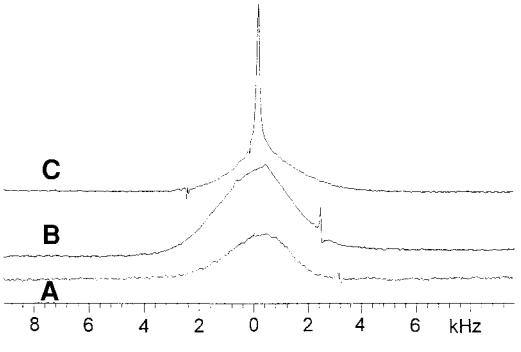

FIG. 2. The Fourier transform of the second half of the echo created by a modified Jeener–Broekart pulse sequence at 1.4 T at 295 K. (A) Dry lyophilized bovine serum albumin. (B) Bovine serum albumin treated with one equivalent of gadolinium(III) ion prior to lyophilization from an aqueous solution. (C) Cross-linked bovine serum albumin gel hydrated to 35% by D_2O.

is that the water signal is very intense, which makes independent characterization of the solid protein component spin relaxation difficult. We have tried to overcome this problem by using D_2O in place of H_2O. The protein was treated with three cycles of lyophilization from excess D_2O to exchange labile protons from NH, OH, and SH sites. The resulting partially deuterated protein is only a model for the completely protonated one and may differ somewhat in the effective internal spin dynamics. For example, because the intramolecular proton concentration is lower, some blocks to proton spin diffusion may be present in the deuterated case that are not present in the protonated case. However, as a model, the partially deuterated protein will relax more slowly than the completely protonated case because the deuterium magnetic moment is smaller than the proton magnetic moment. Thus, we may take the relaxation rates observed in this way as a lower limit to the fully protonated relaxation rate.

A representative decay of the dipolar echo from the hydrated serum abumin gel is shown as curve B of Fig. 3. The dipolar echo amplitude is small compared to the lyophilized case. The decay is more nearly exponential and more than an order of magnitude faster than in the dry protein sample in spite of the deuteration of labile sites. Data for the paramagnetic gels are similar; a representative example is shown in curves A and C of Fig. 3. It is interesting that the relaxation times for the paramagnetic gels are somewhat longer than for the dry systems; however, the decay of the dipolar echo amplitude remains very short, in the tens of microseconds range. An important feature of the hydrated gel samples is that the lineshape of the frequency domain spectrum is altered. The spectrum resulting form a 90° pulse is somewhat distorted by the presence of residual protons in the D_2O. To minimize the direct contribution from the liquid, we use the Fourier transform of the dipolar echo and the resulting spectrum is shown in Fig. 2C. The lineshape is clearly a superposition of broad and narrow component(s). The narrow component is on the order of 5 kHz and is much broader than the water proton signal in similar samples hydrated with H_2O. It is clear that there are regions of the gel that have significant local motion, which lead to a significant motional narrowing of the proton spectrum in these samples with consequent reduction in the values of the dipolar relaxation time.

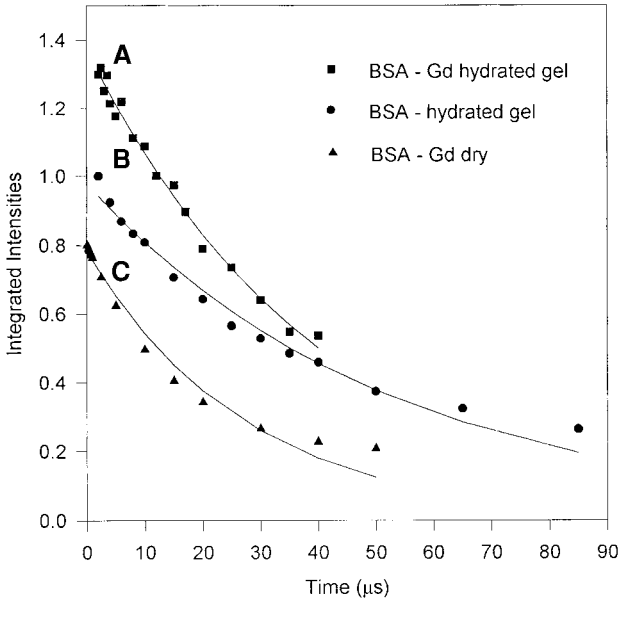

FIG. 3. Echo decay of a Jeener–Broekaert pulse sequence applied to bovine serum albumin at 295 K. (A) (■) Bovine serum albumin gel containing one equivalent of gadolinium(III) hydrated to 35% with D_2O; the exponential time constant for the solid line is 39 μs. (B) (●) Bovine serum albumin gel hydrated to 35% with D_2O; the exponential time constant for the solid line is 52 μs. (C) (▲) Lyophilized serum albumin with one equivalent of gadolinum; the exponential time constant for the solid line is 27 μs.

TABLE 1
Dipolar Relaxation Times at 60 and 500 MHz

	H	D
60 MHz		
BSA	[a]	[a]
Mn-BSA	19 ± 2.5 μs	10 ± 2.5 μs
Gd-BSA	14 ± 2.5 μs	10 ± 2.5 μs
500 MHz		
BSA	[a]	[a]
Mn-BSA	13 ± 2.5 μs	14 ± 2.5 μ
Gd-BSA	12 ± 2.5 μs	14 ± 2.5 μ

Note. H denotes samples lyophilized from H_2O; D denotes samples lyophilized from D_20.

[a] Slow component of decay ≈450 μs ± 50. Fast component: 65–70% of signal intensity.

CONCLUSION

These measurements of the decay of dipolar echoes in diamagnetic proteins, paramagnetic proteins, and hydrated protein gels demonstrate that the dipolar relaxation times in these tissue models are short. In all cases, the measured relaxation times are much shorter than the spin–lattice relaxation times in either protein systems or tissues. Thus, unless there are components that are unusually rigid or crystalline, the dipolar order possibly created by various preparation pulse schemes utilized in the context of magnetization transfer imaging experiments should be transient and have little or no effect on the analysis of the experiment.

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