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High Resolution Q-Space Imaging Studies of Water in Elastin

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Abstract. In this work we report on the direct measurement of the molecular diffusion coefficients of water, confined to purified bovine nuchal ligament elastin, by high resolution q-space NMR imaging. The experimental data indicate that water trapped within an elastin fiber has two distinguishable molecular diffusion coefficients. The component with the slowest mobility has a diffusion coefficient on the order of $10^{-9}$ cm$^2$/s that varies inversely with the diffusion time and is seen to reduce near 37 $^\circ$C. The component with higher mobility has a diffusion coefficient reminiscent of free water but is observed to also behave similarly at 37 $^\circ$C. From our experimental data we extract the surface-to-volume ratio of pores within elastin and associated changes as a function of temperature.

Keywords: elastin; q-space imaging; diffusion NMR;
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INTRODUCTION

Elastin is an extracellular, insoluble, and complex macromolecule that is responsible for the elasticity of many vertebrate tissues such as the skin, lung, and aorta [1]. The elasticity of this remarkable biopolymer is known to be correlated to hydration since the pioneering work of Partridge [2]. This has led many researchers to focus on the complex water-protein interaction by studying the thermal and mechanical properties of elastin in addition to the dynamics of repeating motifs of peptides that mimic its elasticity. Nevertheless, the mechanism resulting in the elasticity of hydrated elastin is still not well understood and several models have been put forward to account for the elastic behavior, all of which implicitly assume waters of hydration [3–6]. In this work, we focus on further understanding of the complex water-elastin interaction by probing the molecular dynamics of water within elastin fibers by q-space NMR imaging [7].

The archetype NMR pulse sequence for these studies involves creating a spatial modulation of the magnetization, termed a grating with a pulsed magnetic field gradient, and then tracking molecular displacements by measuring the attenuation of the grating due to the random motion of the molecules over an experimentally controlled time [8]. The functional form of the signal attenuation as a function of the diffusion time or gradient amplitude is determined by the geometry of the confining pore and the rate of molecular diffusion. For simple geometries, such as spherical or infinite cylindrical pores, q-space imaging allows for a direct measurement of the average pore diameter. When mobile molecules are confined to a porous structure, the measured diffusion coefficient will appear time dependent. In this situation, the attenuation in the signal intensity in short time allows for a measurement of the pore surface to volume ratio, independent of the pore geometry, as shown by Mitra et al. [8]. In the long-time regime, the time dependence in the diffusion coefficient approaches an asymptotic value allowing for a measure of the tortuosity of the system, which characterizes pore connectivity and fluid transport. A challenging aspect of such studies is that spin relaxation causes the net magnetization to decay before the nuclear spins can “see” to full extent the complex connectivity of the system. Thus, probing the tortuosity limit in practice is experimentally difficult due to the fact that signal-to-noise is reduced by relaxation processes if the diffusion time
is made too long. Mair et al. reported on a measurement of the long-time diffusion dynamics of xenon gas imbibed in glass beads allowing for a measurement of the tortuosity of this model system [9]. The advantages in using a gas over a liquid are the diffusion coefficient of gas is much faster, and the relaxation times are much longer and can be controlled to some extent by adjustment of the pressure in the system. Measuring the diffusion coefficient in short time, however, is also challenging due to the fact that the gradient ring down times and eddy currents need to be kept short. Despite these experimental limitations, q-space NMR still lends a great deal of information to the study of complex systems. The experimental challenge for interrogating the internal structure of an elastin fiber, and for probing the rate of molecular motion of waters of hydration by q-space NMR imaging, is that the magnetization grating must have a wavelength less than the diameter of the fiber. Bovine nuchal ligament elastin studied in this work has a diameter approximately equal to 5 µm [10]. To create a grating with a wavelength less than 5 µm, strong pulsed gradient fields in excess of 1 T/cm must be delivered to the nuclear spin ensemble that are not commercially available and were designed in our laboratory.

**FIGURE 1.** (A) Pulsed gradient spin echo (PGSE) and (B) stimulated echo (SE) pulse sequences with the inversion recovery filters. In the experiment, the time Δ₁ is experimentally set to filter the signal arising from bulk water outside the fiber. The time Δ₂ is the diffusion time, which was varied in the experiment. In our experiments, the amplitude of the gradient pulse is also varied, while the gradient pulse length was set to 1 ms. The π/2 pulse width was 3.7 µs, and the recycle delay was set to 10s throughout all our studies.

**EXPERIMENTAL**

For these studies purified bovine nuchal ligament samples were purchased from Elastin Products Company (Elastin Products Co., Owensville, MO) that were prepared by the neutral extraction method of Partridge. The samples were hydrated in deuterated water for one week in a mechanical shaker and included 0.03% (w/v) of sodium azide added as biocide. We detected the ²H signal from deuterated water so that the signal from protons arising from the protein backbone can be easily filtered. The deuterium hydrated samples were placed in a sterile 10 mm by 1.5 mm OD capillary sample tube sealed with epoxy to avoid water loss. We used a modified wide-bore liquids Bruker NMR probe fitted with a home-built gradient set and high power RF capacitors. The gradient coil had a resistance of 1.5 Ohm, an impedance of 25 mH and an efficiency of 400 G/cm/A. The design of such gradient coils for use in solid state NMR scattering experiments is outlined by Zhang and Cory [11]. The RF coil was 2 mm in diameter and consisted of 5 turns of 32 AWG copper wires. We ran the experiments on a Tecmag Apollo NMR spectrometer, which controlled a Techorn 5050 gradient amplifier and delivered up to 50 A of current. In our system, ²H had a resonance frequency of 27.548 MHz. A pulsed gradient spin echo (PGSE) cycle shown in Figure 1A was used to investigate the rate of molecular diffusion of water in the elastin fiber as a function of the wave-number q ranging from 0 to 1.7 x 10⁴ cm⁻¹ and diffusion times Δ ranging from 3 to 12 ms. In addition, a stimulated echo (SE) pulse cycle shown in Figure 1B was implemented to study the rate of diffusion over longer diffusion times by varying q over the same range. Both sequences incorporated a T1 filter to suppress the signal from water outside the elastin fiber, which was determined using a saturation recovery and inversion recovery experiment. Measurements of the T2 were performed using a CPMG cycle at every temperature and indicated the presence of water in two distinct environments within the elastin fiber. The T1 filter is constructed by first accurately measuring the respective T1 relaxation times of the various components of the water within the sample. Assigning the longest T1 to that of bulk or free water, the same T1 inversion recovery filter was implemented in the diffusion studies.
RESULTS

In the diffusion measurements, we found that a model of two exponentially decaying functions fit the data as \( q^2 \) was varied when the diffusion time was less than 8 ms. However, when the diffusion time was made longer than 8 ms a single exponential fit appeared to accurately describe our results. Our rationale for fitting two exponentially decaying functions at short time, indicative of two diffusion coefficients, stems from the T2 observations mentioned earlier. For any given value of D in either the SE or PGSE experiments, it is expected that the signal as a function of \( q^2 \) be described by an exponential or a sum of exponentials depending on the number of environments the water is exposed to. This can be understood as follows: for a porous system having an average pore size \( a \) and a pore spacing \( b \), the signature of scattering events are revealed in the data only when \( q \sim a^{-1} \) or \( q \sim b^{-1} \) [7]. A well-known result from the theoretical treatment of q-space imaging is when \( q \ll b^{-1} \) the echo intensity will decay exponentially as a function of \( q^2 \) [7]. In our experiments, we vary \( q \) over the range of 0 – 0.5 \( \mu m \) and because any pore spacing within the fiber is much less than 1 \( \mu m \), no scattering events are expected to be experimentally realized. In addition, the motion of water molecules confined in the elastin fiber is expected to take a tortuous path because of the complex morphology of the system as revealed in reported SEM studies [12]. As a consequence of the tortuous path of water molecules in the elastin fiber, the diffusive behavior of the water molecules in highly confined regions is expected to be time dependent due to the presence of interconnecting channels that confine their motion. For such a porous system, Mitra et al. showed that for short time that the surface-to-volume ratio can be determined from the relation independent of the confining geometry. For longer times, the measured time dependence in the diffusion coefficients vanishes and the diffusion coefficient is related to the inverse of the tortuosity of the confining structure. To analyze the intermittent behavior between short-time and long-time behaviors, a Padé approximation was introduced by Latour to extrapolate between these two regimes [13]. In Figure 2, the measured diffusion coefficient of the water having a magnitude on the order of \( 10^{-6} \) cm/s, is seen to depend both on the diffusion time and on temperature. In our experiments, we observe changes in the diffusion coefficients over time scales of milliseconds, and the RMS displacement of the \(^2\)H nuclei in a time \( \tau = 4 \) ms with \( D = 10^{-7} \) cm/s is on the order of 1 \( \mu m \). Hence, the observed time dependence in the diffusion coefficients of this component is due to the tortuous path that the water molecules undergo and the slope of the curve in short time is related to the \( S/V_p \) ratio of the channel or pore that confines the motion. In each of the graphs, we show the measured diffusion coefficient as a function of the square root of the diffusion time \( \tau \). The line shown in each graph is a fit to the experimental data whose slope is the \( S/V_p \) of the channel that confines the motion of the molecules. The line fit to the 10 and 20 °C data made use of all the experimental data points shown. For the higher temperatures, we used only the first 4 points in the decay of the diffusion coefficient, which appeared to have a linear behavior with \( D^2 \). An important point that should be emphasized in the linear fitting to the experimental data is that the diffusion coefficient at \( \tau = 0 \) ms is that of free water, which is well known. The graph shows that the fit appears to describe the trend in the experimental data for the 10 and 20 °C quite well, however, not for the 30, 37, and 42 °C data. The physics of these results is that at 10 and 20 °C temperatures, the morphology of the elastin sample is such that the highly confined water molecules traverse a distance where they sample the shape of the confining pore only, and that the tortuosity limit is not reached. The measured surface-to-volume ratio, \( S/V_p \), at these temperatures was \( 1.14 \times 10^{4} \) cm\(^{-1}\) and \( 1.04 \times 10^{4} \) cm\(^{-1}\) for 10 and 20 °C, respectively. At 30, 37, and 42 °C we could not fit the short time limit expression to the all of the experimental data that is shown, suggesting that the dynamics we probed are no longer satisfying this approximation. Careful inspection of \( D(\tau) \) at 30 and 42 °C reveals that the results also show a time dependence at long times from which we deduce that tortuosity limit has not been reached with these measurements. Moreover, the shape of the curve appears to vary in going from 30 to 37 °C and then to 42 °C. The Padé approximation extrapolates between the short and long time limit and requires an estimate of the \( S/V_p \) ratio and the tortuosity of the system in order for other parameters relating to the structure of the pore to be measured. In an ideal experimental condition, we would have shortened the observation time \( \tau \) below 1 ms and probed the short time dynamics of the molecules and more accurately measured \( S/V_p \). Currently, the gradient ring down times and eddy currents in our setup prevents us from doing so. In addition, if the T2 were sufficiently long, we would also probe longer time dynamics of the water and measure \( \alpha \), the tortuosity. In an attempt to study the dynamics and measure the tortuosity of the slow-component in the sample in the long-time regime, we implemented a stimulated echo pulse sequence. The results obtained when \( \tau = 25, 50, \) and 75 ms indicated only 1 diffusion coefficient. We found that the measured values are slightly above those measured by the PGSE cycle for the slow-component shown in Figure 2. At 10 °C the diffusion coefficient measured with \( \tau = 8 \) ms with a PGSE experiment was \( 8.6 \times 10^{-7} \) cm/s, but in the stimulated echo experiment we measured \( D = 1.4 \times 10^{-6} \) cm/s at \( \tau = 25, 50, \) or 75 ms. We believe these results may suggest that over the time scale of 25–75 ms that the highly confined slow-component exchanges with the more bulk-like...
component. A consequence of this exchange prevents us from precisely measuring the tortuosity limit of the slow-component at all temperatures. While further experimental work is necessary to quantify this exchange, here we provide an estimate of the $S/V_p$ ratio from the first few points in the decay for the temperatures above 20 °C. The measured values are $1.02 \times 10^{-6}$ cm$^-1$, $1.04 \times 10^{-4}$ cm$^-1$, and $1.01 \times 10^{-6}$ cm$^-1$ for 30, 37 and 42 °C, respectively.

The changes in the shape of the diffusion coefficient versus the diffusion time curve shown in Figure 2 point to morphology changes within the elastin fiber as the temperature is varied. At the lower temperatures the data appear to be well described by a straight line, in agreement with the leading term of the short time limit. However, at temperatures above 20 °C we find that the diffusion coefficients as a function of $\Delta^{-1/2}$ are no longer linear as measured over the same time scale. Recalling again that elastin has a negative thermal expansion coefficient, an increase in temperature decreases the volume of the entire fiber. Our results, however, do not indicate a significant change in the measured $S/V_p$ as the temperature is raised. The fact that we observe changes in the shape of the diffusion curve as a function of the square root of the diffusion time suggests a complex change in the average micro-pore area and volume within the elastin fiber or that the connectivity of the channels within the elastin fiber may change as a function of temperature. Future work of our laboratory will probe the rate of exchange between the two domains occupied by the waters of hydration and the interplay with protein dynamics and function.

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