Bulk and residual dipolar field effects on the behavior of 1H water signal in tendon and their relation to the three-dimensional structure of the collagen matrix.

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Abstract

Dependence of a T_2^* water signal on tissue orientation in the external magnetic field is used to estimate the strength of residual dipolar interactions. A time-averaged effective direction of such interactions is deduced to lie along the fiber long axis. The residual dipolar field accounts for T_2^* decay at almost all orientations. However, there is an additional mechanism of transverse relaxation at orientation perpendicular to the main field. Shift of the precession frequency is interpreted as a signature of the bulk dipolar field, or the bulk magnetic susceptibility effect. The bulk magnetic susceptibility effect in tendon is reported for the first time and related to the three-dimensional structure of collagen supramolecular assembly. The data reported here is combined with existing findings by X-ray crystallography and electron microscopy to explain behavior of ^{1}H NMR water signal in tendon.

Introduction

Crystallization of collagen macromolecules in extracellular matrix of tissues provides organs with necessary resistance to tensile/ compressive stresses and at the same time creates highly anisotropic media. Anisotropy in such tissues as tendons extends to a macroscopic scale. It is not entirely unexpected that macroscopic order of macromolecules greatly modifies water dynamics in the matrix and contrast in MR images. If origin of such modifications is understood, predictions on the observed signal can be made in cases when normal structural hierarchy of healthy tissues is disrupted.

Methods

Samples of bovine tendon of digital flexor were obtained from the local slaughter house, fresh for each set of measurements. All animals were about 1 year old, sex, diet, injury and load bearing history unknown. Biological variability was ±20% calculated by the unbiased estimate of the data variance. Measurements were done at field strength of 2 Tesla on CSI small animal imager with the horizontal 20 cm bore and Libra Tecmag console, observation frequency 85.6 MHz. Each free induction decay (FID) spectrum was acquired with 28 s duration $\pi/2$ pulse; FID time domain size of 2047 points, 8 averages, 25 μs dwell time, maximum SNR = 400. The beginning of acquisition was delayed by τ= 2.5 ms from the middle of the excitation pulse. To elucidate the effect of the static field inhomogeneity, spin echo data was collected with the Carr-Purcell-Meiboom-Gill sequence with TE of 1.6 ms, 1024 echoes, 8 averages. Reference measurements for registration of frequency shifts were done on a cylindrical vial filled with gadolinium doped water in place of a tendon sample. The temperature was 18.5±0.2°.C. A home-made Helmholtz pair coil with the inner diameter of 5 cm was used as a transmit/receive probe. The sample was positioned on a table in the center of the coil and rotated in increments of 2°-5° with respect to the polarizing field B_0, while in the magnet. Angular precision of mechanical rotation was 1°.

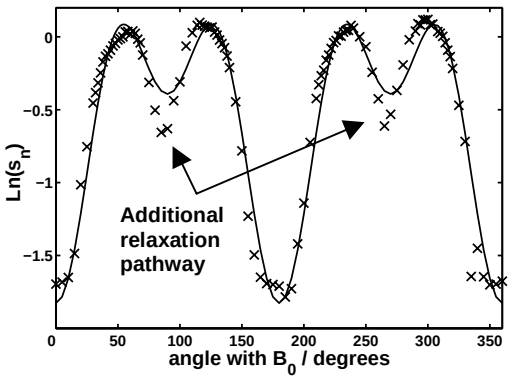

Fig. 1. Orientational dependence of the logarithm of normalized FID amplitude: Crosses (×), experimental data; Solid line (--), fit to the following function: $y=a_1 P_2(\cos\theta)+a_2$; a_1 =-1.913, a_2=0.086.

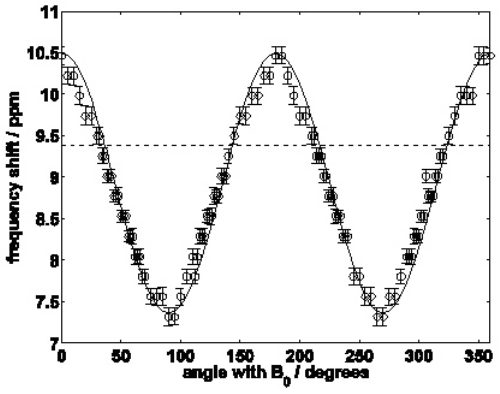

Fig. 2. Central frequency shift as a function of sample orientation, Circles stand for experimentally obtained values; Solid line is a fit to function: $f(\theta)=a_1 y(\theta)+a_2$, where $y(\theta)=(1/2)(3\cos^2((\pi/2)+\theta)-1)$, a_1=-2.08 and a_2=9.45.

Results and Discussion

Figure 1 shows orientational dependence of the natural logarithm of a normalized FID amplitude and a fit to a function $y=a_1P_2(\cos\theta)+a_2$, where $P_2(\cos\theta)=(1/2)(3\cos^2\theta-1)$, θ is an angle between the external magnetic field and the long axis of a specimen, in degrees. In the absence of other relaxation mechanisms, the natural logarithm of the FID amplitude can written as follows: $\ln(s_n(\tau,\theta))=-(1/2)\nu_H^2\tau^2 P_2(\cos\theta)$, where ν_H is the magnitude of the dipolar interaction associated with a pair of protons separated by a distance r, in frequency units, given by $\nu_H=(3/2)(\mu_0/4)\gamma^2\hbar r^{-3}$. Thus, fit coefficients allow estimating ν_H and, then, an effective distance of sensitized interactions. The value of strength of the residual dipolar coupling estimated by this analysis is 780 Hz and agrees well with 900 Hz reported earlier for bovine Achilles tendon by Eliav and Navon. (1). However, it is also seen that at θ=90° there is an additional mechanism left unexplained by the residual dipolar interactions. Figure 2 shows a shift of the precession frequency due to a geometry induced change of the local microscopic field. Effect of boundary geometry was studied extensively before in high resolution NMR to explain frequency shifts and distorted line shapes for non-spherical samples (2). Analysis of orientation dependence of this effect lets us conclude that frequency shifts observed in this study are related to the cylindrical geometry of a cavity oriented perpendicular to the fiber long axis. X-ray crystallography and electron microscopy previously have shown that such cavities indeed exist and are formed by interconnecting gaps in the assembly of collagen molecules into the supramolecular three-dimensional structure known as a fibril. Figure 3 shows a diagram proposed by Landis et al. (3) on the basis of their study on progressive calcification of tendon during the process of normal mineralization of vertebrate tissues.

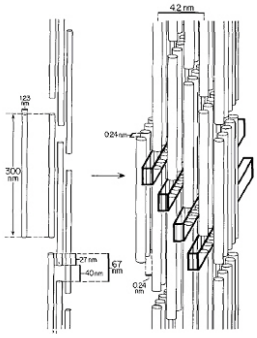

Fig. 3. Packing of collagen in three dimensions occurs such that hole and overlap zones are aligned in strict registration, thereby creating extensive channels or gaps throughout the assemblage.
(Adopted from Landis et al., 1993)

Conclusions

This work describes multifaceted effect of structural anisotropy of the tendon's extracellular matrix on behavior of water signal. The residual dipolar coupling has effective direction along tendon fibers while the bulk magnetic susceptibility effect is related to the cavity transecting the fiber in perpendicular directions. Both effects can be potentially applied to probe the three-dimensional structure of collagen matrix not only in tendon but in other collagen – rich organs and tissues such as bone, cartilage, ligaments, skin, arteries, etc.

References:

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3. Landis WJ, Song MJ, Leith A, McEwen L, McEwen BF. J