Detection of dipolar splitting in rodent tendons as a function axial position with double-quantum filtered spectroscopic imaging

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Introduction

Tendons are comprised of parallel collagen fibers that connect muscles to bone, allowing for transmission of tensile force, and thus facilitating motion. Collagen-associated water has anisotropic rotational motion, which gives rise to residual dipolar splitting in ^{1}H NMR[1]. While not usually observable due to exchange with the large pool of non-collagen associated water, double-quantum filtered (DQF) NMR and MRI can be used to isolate the signal from collagen-associated water to study the biophysical and structural properties of tendon[2]. These methods have been successfully used to study whole tendons *ex vivo* and *in vivo*. However, studies on rodent tendon, which is a common animal model for tendon injury[3], and along the tendon axis, which could provide insight into the tendon-bone insertion site[4], and tissue remodeling due to tendon wrapping around bone[5,6], have not been reported. Here, we modified a DQF 1D spectroscopic imaging sequence[7] to obtain ^{1}H DQF spectra along the length of flexor digitorum profundus (FDP) tendons from rat hind limbs. As the FDP tendon wraps under the calcaneus and sustentaculum tali, the rat's foot will apply pressure on the tendon during locomotion and it is known to exhibit a fibrocartilage-like region (FC) which is characterized by increased proteoglycan content and a patchwork organization of collagen fibers (Fig. 1)[5,6].

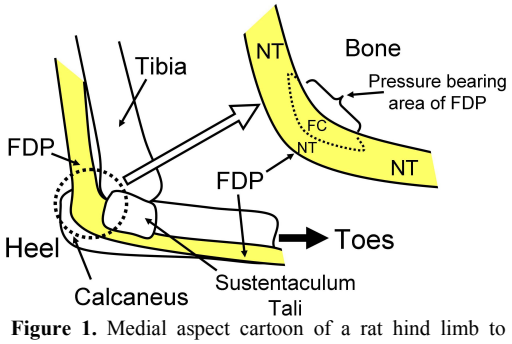

Figure 1. Medial aspect cartoon of a rat hind limb to show the FDP tendon (yellow) in relation to major bones (white). The inset shows a close-up of the tendon-bone interface. Regions of normal tendon proper (NT) and fibrocartilage (FC) are indicated.

Materials and Methods

FDP tendons (~20 *mm* in length) were harvested from 5 healthy male Sprague-Dawley rats (14-24 weeks-old). The specimens were placed flat on a Teflon spatula and inserted in a 5 *mm* NMR tube filled with fluorinated oil (Fomblin, Sigma-Aldrich) to prevent dehydration. The tube was then put in a vertical-bore 9.4T spectrometer/microimaging system (DMX-400, Bruker Instruments) such that the entire FDP tendon was parallel to the z-axis. ^{1}H in-phase (IP) DQF 1D spectroscopic imaging (90°-τ/2-90°-τ_{DQ}-90°-τ/2-90°-τ_{ZQ}-90°-{G_{PE}}-AQC)[7], with the phase-encode gradient (G_{PE}) along the z-axis, was performed with the following parameters: τ=1200μs, 32 PE steps, 0.75 *mm* slice thickness, FOV=24 *mm*. All images were analyzed with Bruker Xwin-NMR software. The temperature was set to 1C to minimize exchange and allow direct observation of the dipolar splitting[2].

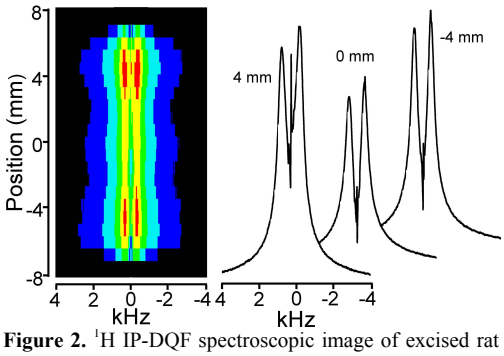

Figure 2. ^{1}H IP-DQF spectroscopic image of excised rat FDP tendon with representative extracted spectra at different axial locations. The x and y axes represent the spectroscopic and spatial dimensions, respectively.

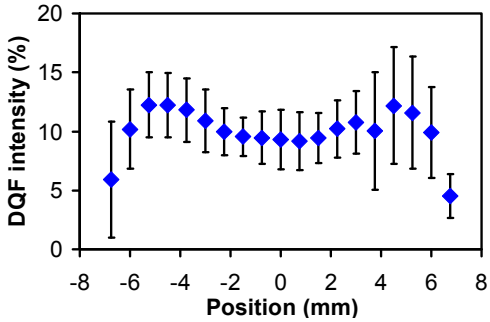

Figure 3. DQF signal intensity as a function of distance from the midpoint of the wrap-around region averaged over all five specimens. Negative and positive distances represent proximal and distal directions, respectively.

Results and Discussion

The IP-DQF sequence was first run without PE gradients on isolated bicep tendons and the dipolar splitting dependence on τ and temperature were reproduced as previously reported[2,7] (not shown). For each FDP tendon specimen, a series of IP-DQF experiments without PE gradients were run to confirm that the maximum DQ signal intensity was acquired at τ=1200μs. Fig. 2 shows a sample ^{1}H IP-DQF spectroscopic image of a FDP tendon. Note the decreased intensity in the center of the image, which corresponds to the region of the tendon that wraps under the calcaneus. The central peak in the extracted spectra is the result of single-quantum leakage. Fig. 3 shows the DQ signal intensity (as measured by the DQF spectral area divided by the pulse-acquire spectrum area) as a function of distance from the midpoint of the wrap-around region averaged over all specimens. The decrease in DQF intensity observed at the ends of the graph is the result of reaching the edge of the sensitivity volume of the RF coil. No significant change in splitting was observed over the tendon length (mean $\Delta\omega$ = 748±36 Hz). The variation in splitting was due to uncertainty in the measurement of the splitting of the relatively broad peaks ($\Delta\nu$ ~ 500 Hz).

No observed change in splitting despite a decrease in DQ signal intensity is inconsistent because splitting and intensity both reflect the strength of the residual dipolar interaction[2,7]. Referring to Fig. 1, it can be seen that the FDP tendon only has a FC region on the side in contact with bone – the opposite side still exhibits characteristics of normal tendon (NT), i.e. parallel collagen fibers and low proteoglycan content. As the splitting does not change along tendon length, this suggests that the DQ signal is only coming from NT and since the NT volume fraction decreases in the wrap-around region, the DQ signal will decrease as a result. Implicit is that no DQ signal is detected from the FC region, which most likely is the result of the patchwork organization of the collagen fibers coupled with the complex dependence of the dipolar interaction on the location, as well as exchange with, neighboring protons.

Conclusion

The work shows that the IP-DQF 1D spectroscopic imaging sequence is suited for studying DQF spectra along the length of tendons and that it can differentiate known changes in tissue architecture along the axis of the rat FDP tendon due to compressive forces.

References: 1. Migchelsen et al, *J Chem Phys*, **59**:296 (1973). 2. Navon et al, *JMRI*, **25**:362 (2007). 3. Soslowsky, et al, *JSES*, **5**:383 (1996). 4. Genin et al, *Biophys J*, **97**:976 (2009). 5. Vogel et al, *Int Rev Cytol*, **115**:267 (1989). 6. Merrilees et al, *Am J Anat*, **157**:87 (1980). 7. Navon et al, *NMR Biomed*, **19**:877 (2006).
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