Cortical bone water studied with ^{1}H and ^{2}H double-quantum filtered NMR

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Introduction

NMR and MRI are powerful tools for non-destructive study of bone water, which plays a pivotal in providing bone with high impact strength. Bone water occupies pores, which consist of an elaborate network of channels that establish communication between the blood supply and osteocytes (Haversian and lacuno-canalicular system) covering a size scale ranging from 0.1 to 100µm. Another significant fraction of bone water is associated with the collagen matrix. This portion of water has anisotropic rotational motion and thus is expected to give rise to residual dipolar and quadrupolar splittings in the ^{1}H and ^{2}H spectra, respectively. A quantitative assessment of cortical bone water, and thus porosity, requires an understanding of the various contributions to the overall MR signal. The difficulty in studying collagen-associated water is that the overall signal is dominated by pore water. Here, we studied the MR signal from collagen-associated water with ^{1}H and ^{2}H in-phase double-quantum filtered (IP-DQF) NMR, which filters out the dominant pore water signal based on the anisotropic rotational motion of collagen-associated water, as previously used to study collagen fiber orientation in tendon and articular cartilage[1].

Materials and Methods

Cortical bone specimens (4×3×15 mm^3) were harvested from the tibia mid-shafts of healthy 6-8 month old lambs. Upon removal from storage (for at least 24 hrs) in saline or 99.8% D_2O, depending on whether ^{1}H or ^{2}H experiments were to be performed, surface water was removed by brief padding with tissue paper. The specimens were then placed in a 5mm NMR tube in a vertical-bore 9.4T spectrometer (DMX-400, Bruker Instruments). ^{1}H and ^{2}H pulse-acquire and IP-DQF experiments (90°-τ/2-90°-τ_{DQ}-90°-τ/2-90°-τ_{ZQ}-90°-AQC)[1] were run with the following parameters: ***^{1}H pulse-acquire*** – 256 scans, τ_{90} = 3.4 μs, TR=2*s*, SW=200kHz; ***^{1}H IP-DQF*** – 256 scans, τ_{90}/ τ/ τ_{DQ}/ τ_{ZQ} = 3.4/25/5/5μs, TR=2*s*, SW=200kHz; ***^{2}H pulse-acquire*** – 512 scans, τ_{90} = 35 μs, TR=0.5*s*, SW=100kHz; ***^{2}H IP-DQF*** – 512 scans, τ_{90}/ τ/ τ_{DQ}/ τ_{ZQ} = 35/135/37/37μs, TR=0.5*s*, SW=100kHz. The sample temperature was varied from 5 to 50°C. All spectra were analyzed with standard Bruker Xwin-NMR software.

Results and Discussion

Fig. 1 shows representative ^{1}H and ^{2}H pulse-acquire and IP-DQF spectra with the longitudinal axis of a D_2O-equilibrated bone specimen aligned parallel and perpendicular to B_0. A strong central peak arising from pore water, subsequently removed in the IP-DQF spectra, can be seen in the pulse-acquire spectra. The ^{1}H pulse-acquire and IP-DQF spectra of saline-equilibrated bone specimens were similar to that shown in Fig. 1 (not shown). The persistence ^{1}H dipolar splitting after equilibration with D_2O and invariance over the range of temperatures (not shown) suggests the ^{1}H splittings to arise from non-labile protons on the collagen backbone. Invariance of the ^{1}H dipolar splitting to specimen orientation would be expected as collagen protons do not have a preferential orientation. Only the ^{2}H quadrupolar splittings showed angular dependence suggesting a preferential alignment of the collagen fibers along the osteonal, i.e. the loading, direction. ^{2}H quadrupolar splittings are considerably larger than those found in cartilage and tendon[1] and may be due to increased collagen rigidity in bone.

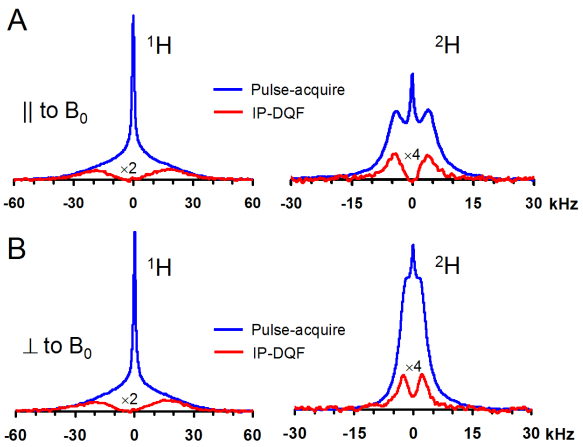

Figure 1. Pulse-acquire and IP-DQF spectra with the longitudinal axis of a D_2O equilibrated lamb specimen aligned (A) parallel and (B) perpendicular to B_0. At these specific creation times, only ^{2}H splittings show angular dependence (8 and 4.7 kHz), while ^{1}H splittings were invariant at ~40 kHz.

Fig. 2 shows representative ^{1}H and ^{2}H IP-DQF spectra of a saline- and a D_2O-equilibrated bone specimen, respectively, at various creation time values, τ. The creation time controls which splittings (Δω) the IP-DQF sequence accentuates based on the dependence of the doublet peak intensity on sin(τ×Δω). The central peak in the ^{1}H IP-DQF spectrum is the result of single-quantum (SQ) leak-through. The ^{1}H splitting clearly decreases with increasing τ, which suggests that there exists a broad range of dipolar splittings, which would be expected for collagen protons due to the variation in inter-nuclear distances and angles. The ^{2}H splitting does not show the same strong dependence on τ, which suggests that there is a single dominant quadrupolar splitting arising from the highly oriented collagen-associated water population. At long enough τ, the IP-DQF sequence acts as a SQ filter, as observed in the disappearance of splittings at long τ values in both ^{1}H and ^{2}H IP-DQF spectra.

The lack of observation of ^{1}H dipolar splittings from the collagen-associated water may be due to the complex dependence of the dipolar interaction on the location, as well as exchange with, neighboring protons. Quadrupolar splittings are more readily observable as the quadrupolar interaction has no dependence on neighboring deuterons and leads to inherently larger splittings. While dipolar splittings have been observed for collagen-associated water in tendons[1], the collagen fiber organization in bone is more complex and exhibits a plywood-like structure[2]. The effect of this increased complexity of collagen fiber organization on dipolar and quadrupolar splittings is unclear and needs further investigation.

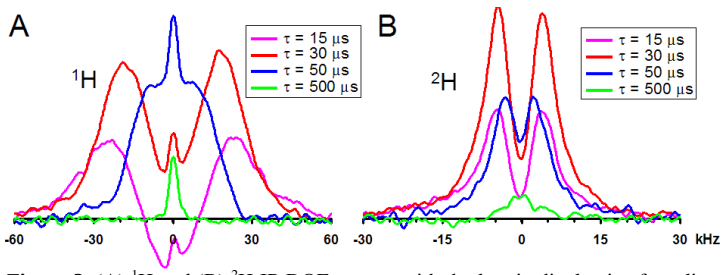

Figure 2. (A) ^{1}H and (B) ^{2}H IP-DQF spectra with the longitudinal axis of a saline equilibrated lamb specimen aligned parallel at various creation times, τ.

Conclusion

To the best of our knowledge, dipolar and quadrupolar splittings from bone have been reported here for the first time with ^{1}H and ^{2}H IP-DQF NMR. IP-DQF may provide insight into collagen fiber organization and the role of collagen-associated water to the overall bone MR signal.

References: 1. Navon et al, *NMR Biomed*, **19**:877 (2006). 2. Giraud-Gille et al, *Calcif Tissue Int*, **42**:167 (1988). **Acknowledgements:** NIH R01 AR50068