NMR Study of the Dynamic Properties of Bone Water

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Introduction

Water plays a pivotal role in the biology of bone. It serves as a solvent for transport of nutrients to and from osteocytes and largely determines the bone's viscoelastic properties. Despite its importance, the nature and binding properties of bone water are incompletely understood. Fernandez et al [2,3], by studying the H_2O/D_2O exchange kinetics, were able to measure water diffusion in solid bone, suggesting the presence of two water components of widely differing diffusion rate. In this work we attempted to obtain more detailed information on the properties of the two fractions by monitoring the NMR signal while the water is gradually expelled by dehydrating the bone at elevated temperature.

Methods and Materials

The sample was a rectangular section (10×4×1mm) of cortical bone, with the marrow removed, harvested from the mid-shaft of the tibia of a 14 week old New Zealand white rabbit. Upon removal from storage in brine (to prevent leaching of minerals), surface water was removed by brief padding with tissue paper. Thereafter, the sample was placed in a 5mm NMR tube with no lid in a vertical-bore spectrometer operating at 9.4T (DMX-400, Bruker), heated to 100°C and maintained at this temperature for 48 hours. After 20 minutes (to allow equilibration) the FID was acquired (30 scans, 20° flip angle = 1.72μs, T_R = 1.5s, dwell time = 5μs) over 48 hours (every 5, 15 and 60 minutes for time periods 0-1, 1-6 and 6-48 hours). T_1 was measured with inversion recovery (τ_{90} = 7.75μs, τ_{180} = 15.5μs, T_I = 0.1 – 3s) at various times throughout the 48 hours (Table 2). Each FID was fitted to a biexponential function of the form $M_1 \exp\{-t / T_2^{*(1)}\} + M_2 \exp\{-t / T_2^{*(2)}\}$ in a least squares fashion (Figure 1) and the magnitudes of each component (M_1 and M_2) plotted as a function of drying time. Diffusion constants were calculated by modelling the bone as a one-dimensional object of length d (the thickness of the tibia) and diffusion constant, D, with no impedance at the boundary and infinite diffusion constant outside the sample (to model steam). In this case the signal, $I(t)$, at long times is given by $I(t) \approx 8I(0)/\pi^2 \exp\{-D\pi^2 t / d^2\}$ [4]. Finite element simulations were run based on the same one dimensional model using the calculated values of D and $I(0)$ (Figure 2).

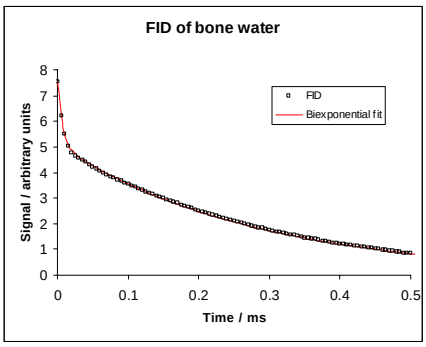


Figure 1 FID of bone water at 100°C

Component	D / 10^{-7} cm^2s^{-1} 1st stage	2nd stage
Total signal	6.50	0.45
Short T_2^*	1.83	0.27
Long T_2^*	7.17	0.50

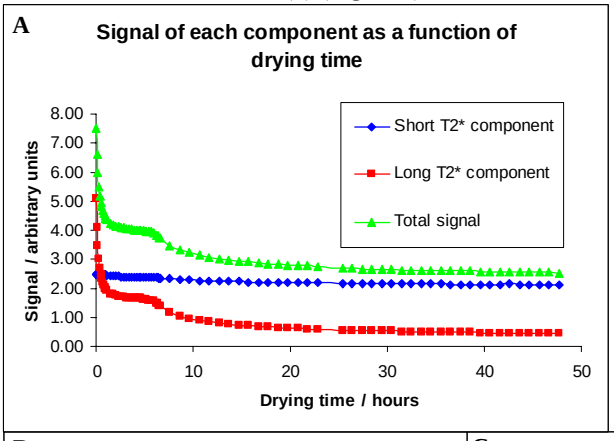
Table 1 Diffusion constants of each water component each stage of the drying process.

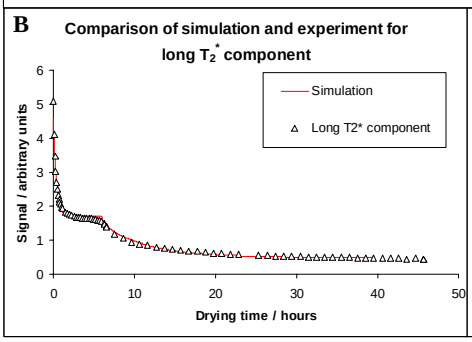
Time / hours	T_1 / s Short T_2^*	Long T_2^*
0	0.841	0.8397
5.6	0.9865	0.9855
21.5	1.2149	1.2019
27.4	1.2899	1.329
45.5	1.3656	1.4532

Table 2 T_1 of remaining bone water as a function of drying time at 100°C.

Results and Discussion

All the FIDs decay in a biexponential fashion with T_2^* = 6.5 and 250μs (Figure 1). T_1 was similar for both components and showed a general increase (from 840 to 1400ms) with drying time (Table 2). This change reflects the reduced H_2O – H_2O interactions as the water content decreases. All the decay curves (Figure 2) followed a two-stage behaviour with the transition point between the two stages being around 6 hours. Despite this unexpected result, the decay of each stage is approximately mono-exponential enabling diffusion coefficients to be calculated using [4]. The results (Table 1) show that the long T_2^* component is more mobile than the short T_2^* component, consistent with their assignments as free and bound water respectively. Simulations using these values of D are in good agreement with experiment for each stage and each component (Figure 2 B,C). It is likely that the two-stage behaviour is dependent on complex binding properties of water and is suggested that it could involve collagen denaturing, a process that occurs around 70°C.





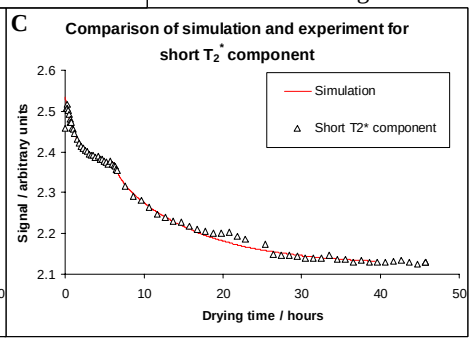


Figure 2 A bone water content as a function of drying time for each component. **B, C** time course of long (**B**) and short (**C**) T_2^* components compared with simulations.

Conclusions

Time-resolved NMR experiments during drying of bone can provide insight into the dynamics of water in different binding states.

References

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