The organic-mineral interface in bone is stabilized by polysaccharides: {31P} – 13C REDOR solid state NMR evidence

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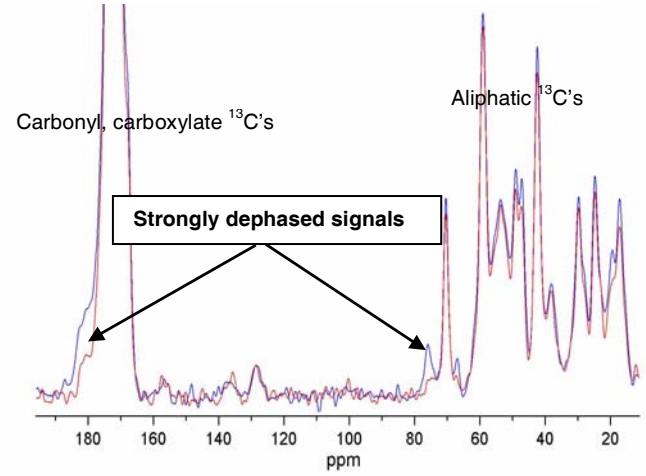
Bone is a complex composite of organic and mineral phases which confer material properties of toughness and hardness respectively. The organic phase is a matrix of proteins, mainly collagen, and other macromolecules including proteoglycans (PGs) rich in acidic glycosaminoglycan (GAG) polysaccharides. The inorganic phase is a hydroxylated calcium phosphate resembling the mineral hydroxyapatite. Although the relationship between the two phases must be crucial to the properties of bone in healthy and diseased states, little is known about the macromolecules responsible for stabilising the interface. Conjecture has centred round a role for acidic proteins. The solid state NMR technique known as Rotational Echo Double Resonance (REDOR)[1], and the abundance of ^{31}P nuclei in bone mineral, offer a unique probe of the atomic level structure of the organic-mineral interface. REDOR employs rotor-synchronised ^{31}P π pulses to reintroduce the ^{31}P – ^{13}C dipolar coupling while observing ^{13}C. Only signals from ^{13}C nuclei less than 4 to 5 Å from phosphorus atoms in the mineral component will dephase, and their intensity will decrease.

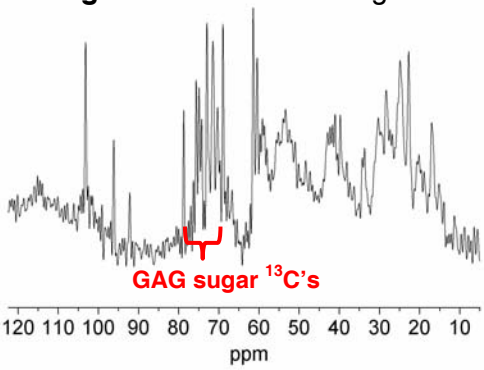
Experimental Tissues were obtained from horses euthanised for purposes other than this study. Spectra were recorded on a Bruker AVANCE 400 three RF-channel spectrometer using high speed MAS. The experimental principles of {^{31}P } – ^{13}C REDOR applied to bone have been described[2] and were followed in this study.

Figure 1 Bone {^{31}P}-^{13}C REDOR



Results Figure 1 shows overlaid {^{31}P } – ^{13}C REDOR spectra from powdered equine bone acquired without (blue trace) and with (red trace) a train of rotor synchronized ^{31}P π pulses of 8 ms duration. Many of the signals in the ^{13}C spectrum can be assigned by analogy with the spectrum of Type I collagen[3] which forms a high proportion of the organic component of bone. The signals which are most prominently dephased by ^{31}P in the REDOR experiment are at chemical shifts of 180-185ppm and centred at about 77 ppm (arrowed). The former shift range suggests carboxylic acid groups, but the latter is not consistent with any common amino acid residue in a diamagnetic environment, or even the γ-carbons of hydroxyproline (random coil shift of 71 ppm) or γ-carboxyglutamate (55 ppm). To test whether the 77 ppm signal may arise from sugar residues in PGs we obtained a ^{13}C MAS spectrum from equine articular cartilage, which is much richer in GAGs than bone. The spectrum (Figure 2) has prominent signals between 70 and 78 ppm consistent with assignments of the ^{13}C resonances of common cartilage GAGs such as the chondroitin sulfates[4]. Although their signals are much sharper in the more liquid-like environment of cartilage, we propose it is these biomolecules producing the broad signal at 77 ppm in the bone spectrum which is so effectively dephased by ^{31}P in the bone mineral. Furthermore carboxylate groups in the GAGs will contribute to the 180-185ppm signal and also dephase if involved in mineral binding.

Figure 2 Articular cartilage ^{13}C



Discussion There is circumstantial evidence for a role for GAG sulfates in the formation and structural integrity of bone; for instance the brachymorphic mouse, which is deficient in sulfated chondroitin, shows abnormally massive and crystalline bone mineral[5]. This work is the first demonstration of an intimate natural association between bone mineral and GAG, and suggests that the primary macromolecules stabilizing the mineral-organic interface are predominantly glycans rather than proteins. This may have implications for the etiology and treatment of bone diseases like osteoporosis.

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