The Organic−Mineral Interface in Teeth Is Like That in Bone and Dominated by Polysaccharides: Universal Mediators of Normal Calcium Phosphate Biomineralization in Vertebrates?

David G. Reid,[†] Melinda J. Duer,[†,*] Rachel C. Murray,[‡] and Erica R. Wise.[†]

Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom, and Animal Health Trust, Lanwades Park, Newmarket, Suffolk CB8 7UU, United Kingdom

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Teeth of mammals and many other vertebrates are made up of the three distinct substances, enamel, dentin, and cement.[1] Cement is in many respects modified bone; it occurs as a thin film attached to the outside of dentin and holds the collagen fibers of the periodontal ligament. Enamel provides the hard surface of teeth and is about 97% apatitic mineral. Dentin, the major constituent, is a less mineralized material, extremely tough and impact resistant. The mineral is apatite-like and is bound into an organic matrix in which collagen is prominent. As in bone, the relationship between these two phases must be fundamental for toughness and hardness. We have shown that the solid-state NMR (SSNMR) technique called rotational echo double resonance (REDOR)[2] is a powerful tool for studying such interfaces in calcium phosphate biominerals.[3] This is because practically all the phosphorus is confined to the mineral and all the carbon to the organic phases, so the $^{13}C\{^{31}P\}$ REDOR experiment, which restores the through-space dipole−dipole coupling between nuclei of the two elements, is highly selective for the biomolecules at the boundary layer. Using this approach, we have shown that the molecules in bone most strongly associated with the interface are sugars, probably acidic glycosaminoglycans (GAGs), and not collagenous or other proteins as is widely assumed.[4] Their prominence at the boundary argues an important role in controlling the formation and properties of the composite material, perhaps by directing calcium phosphate solidification and preventing inappropriate overcrystallization. Here, we report that the organic−mineral interface in teeth is very similar to that in bone and also dominated by polysaccharides.

Figure 1 compares SSNMR cross-polarization magic-angle spinning (CPMAS) experiments carried out on powdered dental material and bone.[6] At the shortest dephasing time explored (3.2 ms), $^{13}C\{^{31}P\}$ REDOR effects are most evident on two low-field signals at ca. 175 and 182 ppm, consistent with amide and carboxylate carbonyl carbons, a signal at 76 ppm attributable to the majority of the ring signals of GAGs (although this signal in tooth has also been ascribed to an anomalously shifted hydroxyproline γ-carbon[7]), as well as minor effects to signal envelopes between 50 and 60 ppm, which could correspond to GAG components such as amidated ring carbons, as well as certain amino acids. At longer dephasing times (such as the 9.6 ms shown), more widespread effects are evident that are very similar to those obtained in bone, whereas the GAG ring signal at 76 ppm dephases to baseline, showing that practically all GAG is in tight association with mineral. Attempts to perform REDOR on a sample of enamel chipped from the upper surface of a tooth failed to give meaningful data because of the extremely low organic content of the material. Nevertheless, the spectral profile is very similar to that of whole tooth, and bone. On the basis of the extremely weak signal from enamel, we

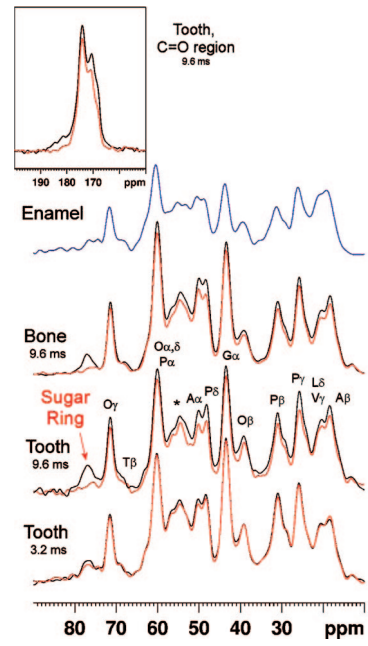

Figure 1. ^{13}C CPMAS SSNMR spectra of tooth material and bone. The bottom three overlays correspond to data acquired without (black) and with (red) $^{13}C\{^{31}P\}$ REDOR implemented on whole powdered tooth, and bone. The inset shows the REDOR effects on the low-field carbonyl/carboxylate signal envelope and includes an effect (at the high-field shoulder) to mineral carbonate. REDOR buildup is too slow to be attributable to intramolecular effects arising from, for instance, phosphoproteins.[3] Assignments shown are based on chemical shifts of the amino acids constituting type I collagen.[5] (A, alanine; G, glycine; L, leucine; O, hydroxyproline; P, proline; T, threonine; V, valine).

* Corresponding author. E-mail: mjd13@cam.ac.uk.
† University of Cambridge.
‡ Animal Health Trust.

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(6) Bruker AVANCE 400, MAS rate 12.5 kHz, contact time 2.5 ms, repetition time 2 s, $^{13}C\{^{31}P\}$ REDOR performed with ^{31}P π pulses applied every 80 μs with a ^{13}C π refocussing pulse at the centre of the ^{31}P pulse train, under 70 kHz ^{1}H broad band decoupling. Total acquisition times varied according to the organic content of the material and were typically 1−3 days for a REDOR experiment on bone or bulk tooth material, or 6 days for enamel. All samples were harvested from horses euthanased for humanitarian reasons unconnected with this study.

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assume that the signal from bulk tooth, which in horses contains enamel involuted with dentin, is in fact very largely from the latter.

There has been much discussion of the role of proteins in directing the orderly mineralization of hard tissues,[8,9] inspiring several reports of NMR experiments to characterize the relationship between mineral surface and the conformations, dynamics, and contact points of bound model peptides.[10,11] It is perhaps time to direct more attention to the role of GAGs, however, as $^{13}C\{^{31}P\}$ REDOR effects similar to those observed in other biominerals have been reported in model composites formed from calcium phosphates and GAGs.[12] Although this communication is the first demonstration of an intimate physical association between mineral and GAGs in teeth (as there is in bone), there is a wealth of circumstantial evidence implicating GAGs in the mineralization process. Indeed, there has been considerable effort to characterize the glycosylation patterns of many of the proteins invoked in the tooth mineralization process, such as amelogenin[13] and perlecan.[14] Changes in GAG composition of the glycoside components of biglycan and decorin have been studied across the transitional boundary between unmineralized predentin and mineralized dentin. The affinities of the GAGs, which are predominantly chondroitin sulfates, for hydroxyapatite increases considerably, on the basis of which participation of GAGs in tooth biomineralization has been inferred.[15] A direct correlation between GAG content and mineralization has been shown in developing rat dental material,[16] while keratan and dermatan sulfate proteoglycan participation has been invoked in both dental calcification and matrix formation.[17] Using chondroitinase treatment and electron microscopy an intimate association between GAG and mineral in dentin has been deduced; in fact these authors present a model of GAG associated with collagen (via associations of small leucine rich repeat proteins, SLRPs) in which mineralization is explicitly depicted as occurring in the neighborhood of the GAG chains.[18]

In modern mammals, there is clear distinction between the materials comprising tooth and bone, although the mechanical properties of each are not dissimilar. For instance, once laid down, dentin is incapable of remodelling; this is unlike bone, in which this is possible thanks to a rich vascular supply. Having observed the close association of GAGs with mineral in numerous samples of bone and other calcified connective tissues, from different anatomical locations, species, and ages, and now in tooth, a material that lies at the opposite end of a spectrum of biomineral types, we hypothesize that GAGs are universal participants in nonpathological formation of biominerals based on calcium and phosphate.

The inability of dental tissue to regenerate spontaneously has prompted much effort in the design of artificial scaffolds in which odontogenesis can be promoted, using established approaches of tissue engineering.[19] These typically employ biodegradable scaffolds based on materials like polyglycolic acids or collagen sponges as a framework for the proliferation of tooth progenitor cells.[20] The discovery that it is GAGs which are most intimately associated with dental mineral in natural tooth argues that it will be important to include GAGs in any strategy aimed at restoring or mimicking tooth material.

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