Probing the Earliest Phases of Bone Mineralization using Attenuated Total Reflection Infrared Microspectroscopy

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The process of bone mineralization is a poorly understood process. It is known that collagen deposition and cross-linking are necessary precursors to mineralization, but the method by which the collagen matrix "seeds" crystallization is unclear. Mature bone mineral is described as highly substituted hydroxyapatite, but there is a long-standing controversy about the nature of the earliest state of bone mineral. Two primary candidates for new bone mineral are amorphous calcium phosphate (ACP) and octacalcium phosphate (OCP). The formation of either of these phases of solid calcium phosphate, however, would be unstable and rapidly convert to hydroxyapatite. Thus, identifying the first bone mineral crystals is a difficult task. Intramuscular herringbones have unique mineralization properties; one end of the bone is completely mineralized and the opposite end of the bone is non-mineralized. In this study, attenuated total reflection infrared microspectroscopy is used to examine the mineral composition of the herring bone near the point where mineralization begins. In order to "capture" the (unstable) earliest mineralization phase, a diamond Schwarzchild ATR objective is used for examining the whole bone, without the need to embed and section the sample. The combination of a diamond ATR crystal and an MCT-B detector permits analysis of the important ν_4 phosphate contour for the first time. Using a 10x10 micron aperture, infrared spectra are collected in 10 micron steps along the length of the bone. **Figure 1** illustrates that the $\nu_{1,3}$ (900-1200 cm^{-1}) and ν_4 (500-650 cm^{-1}) phosphate contours increases in intensity along the length of the bone, indicating an increase in bone mineralization. At the lowest mineralization observed in this map (~2% of maximum), the phosphate contour slightly resembles that of poorly crystalline biological apatite. However, clear differences exist, notably an intense feature at ~520 cm^{-1}, which has been attributed to HPO_4^{2-} in the crystal lattice. In addition, the normal ν_4 apatitic phosphate modes are shifted to lower frequency, suggesting a unique phosphate environment in these early mineralization stages.

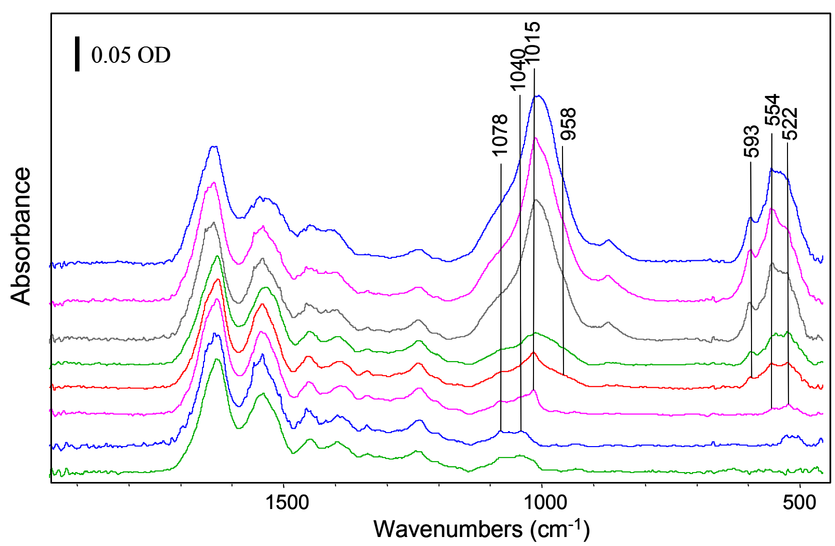

Figure 1. Infrared spectra of intact herring bone using ATR-IR microspectroscopy. Data were collected from one end of the bone to the other, which corresponds to the least and most mineralized regions, respectively. The spectra shown above were collected in 10 micron steps. Of all spectra are normalized to the Amide I (protein) content to illustrate changes in mineralization.