Natural-Abundance ^{43}Ca Solid-State NMR Spectroscopy of Bone

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Raman spectroscopy

All Raman experiments were performed using a locally constructed Raman microscope as described elsewhere.[1] The major components of this instrument are a 785 nm diode laser (Inivctus, Kaiser Optical Systems, Inc., Ann Arbor, MI), a Nikon E600 epi-fluorescence microscope fitted with a 20x/0.75 NA S Fluor objective (Nikon, Inc., Melville, New York), an axial transmissive spectrograph (HoloSpec Kaiser Optical Systems, Inc.) and a 128x1024 back-illuminated deep depletion CCD detector (DU401-BR-DD, Andor Technologies, Belfast, Northern Ireland). The laser was line-focused onto bone specimens, and the Raman back-scattered light was collected through the objective, dispersed through the spectrograph at 3-4 cm^{-1} resolution and integrated for 10

min on the CCD. The resulting 126 spectra were arranged at equidistant points along a line through the specimen. Raman data were processed in MATLAB software (v. 7.0, The Math Works, Natick, Massachusetts) with corrections for cosmic ray spikes, image curvature, dark current, and variations in the CCD quantum efficiency.

Raman spectroscopy indicates the similarity between synthetic carbonated apatite and bone mineral

Raman spectra of bovine bone and related materials are shown in Figure S1. All spectra have a strong PO_4^{3-} band at 958 cm^{-1} and are normalized to the height of this band. Figure 1a is a typical cortical bone Raman spectrum with phosphate bands (at 430 cm^{-1} and 587 cm^{-1}, and PO_4^{3-} symmetric stretch at 958 cm^{-1} and asymmetric stretch at 1075 cm^{-1}) and carbonate (at 1070 cm^{-1}) and matrix bands, predominantly collagen, at (853, 875, 920, 1003, 1267, 1451 and 1667 cm^{-1}).[1] The matrix bands are absent from the Raman spectrum of deproteinated bone (Figure 1b) confirming hydrazine removal of at least 95% of the organic matrix. Figure 1c shows the Raman spectrum of synthetic carbonated apatite, with a carbonate band at 1070 cm^{-1}. Only the phosphate bands are found on the Raman spectrum of hydroxyapatite (Figure 1d).

The full-width at half-height (FWHH) of the phosphate band at 958 cm^{-1} (bone, 19.4 cm^{-1} and deproteinated bone, 19.2 cm^{-1}; compared to synthetic carbonated apatite, 14.7 cm^{-1} and hydroxyapatite, 10.0 cm^{-1}) confirms that bone mineral has low crystallinity, as expected.[2,3] The unchanged band positions and FWHH confirm that deproteination causes a little or no change to the mineral. Synthesized carbonated apatite was found to be more crystalline than the natural mineral, as expected because it does not have the

extensive range of substituents (Mg^{2+}, F^-, etc.) that are expected in the bone and because crystallites are larger than bone mineral crystallites. Finally, the stoichiometric hydroxyapatite is known to precipitate with crystals that can be 1 μm long or longer and without extensive substitution by other cations or anions, resulting in a smaller FWHH than found in the other materials examined. Structurally, synthetic carbonated apatite is more similar to bone mineral than is synthetic hydroxyapatite and is widely used as a model for bone mineral.

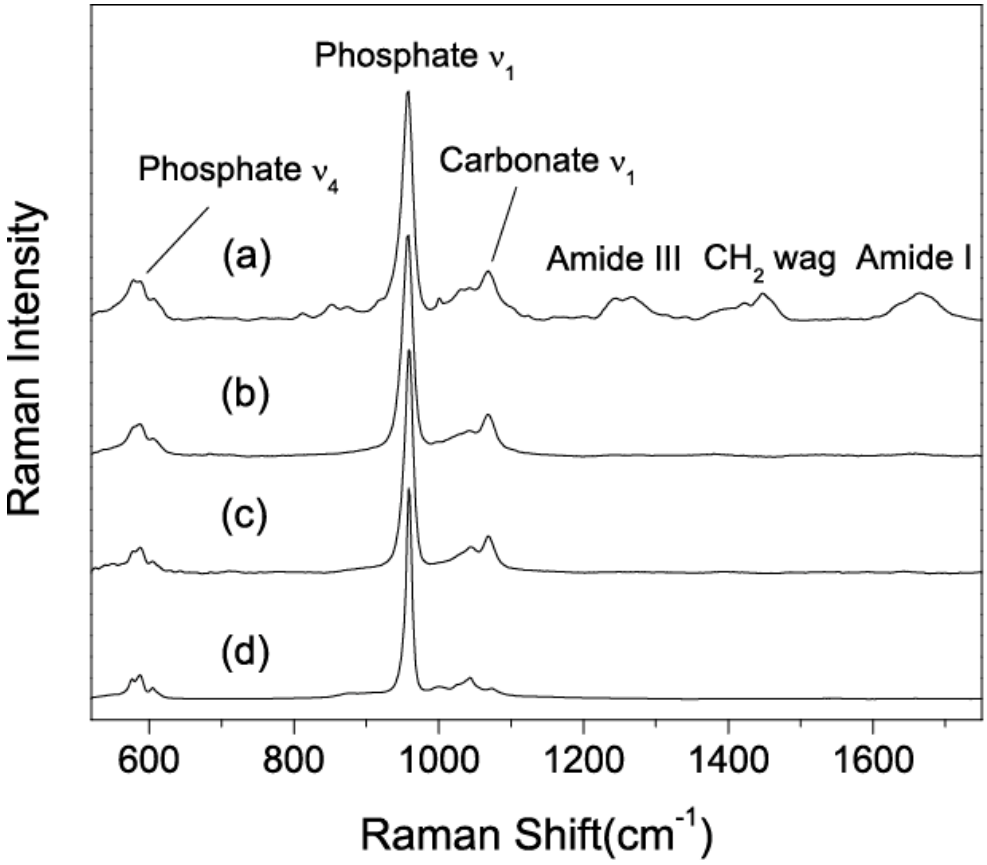

Figure S1. Raman spectra of bone and model compounds: (a) cortical bone, (b) deproteined cortical bone, (c) carbonated apatite, (d) hydroxyapatite. All spectra were

obtained using a Raman scatter system excited by a 785-nm laser with a rectangular beam profile (Kaiser Optical Systems) and passed through a ×20 objective that focuses the line-shaped beam (100 μm in length). Raman scattered light from every point on the line was simultaneously passed back through the objective and through a dichroic mirror to a charge coupled device detector.

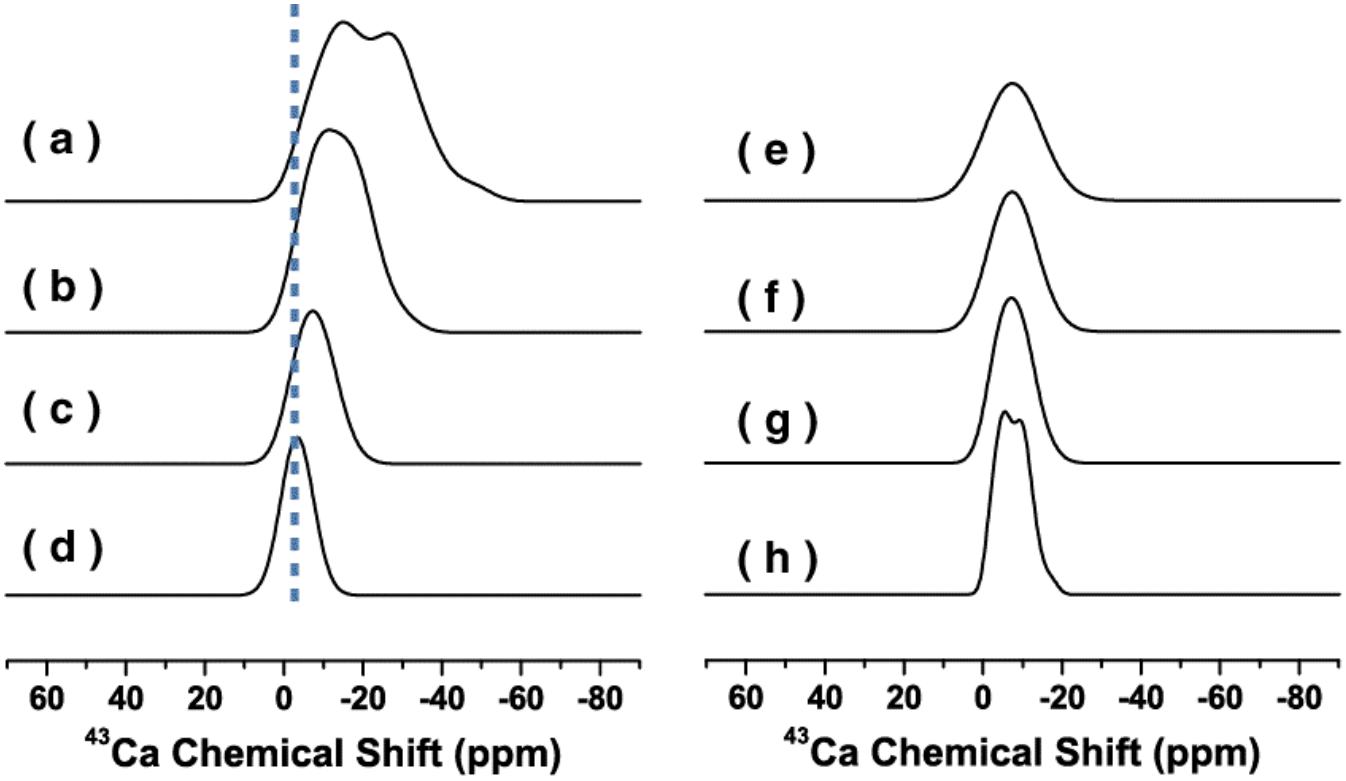

Figure S2. Determination of ^{43}Ca NMR parameters using simulations. Simulated ^{43}Ca NMR spectra with different quadrupole coupling constant C_Q and line broadening

values, while the chemical shift (0 ppm) and asymmetry parameter, η_Q=0.4, values were not varied. In the first column, spectra generated from the use of a 700 Hz line broading along with C_Q= 5 MHz (a), 4 MHz (b), 3 MHz (c) and 2 MHz (d) values. C_Q= 3 MHz was used to obtain spectra in the second column along with a line broadening of 800 Hz (e), 600 Hz (f), 400 Hz (g) and 200 Hz (h).

References

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