

Supporting Information

Regulation of calcium phosphate formation by amelogenins under physiological conditions

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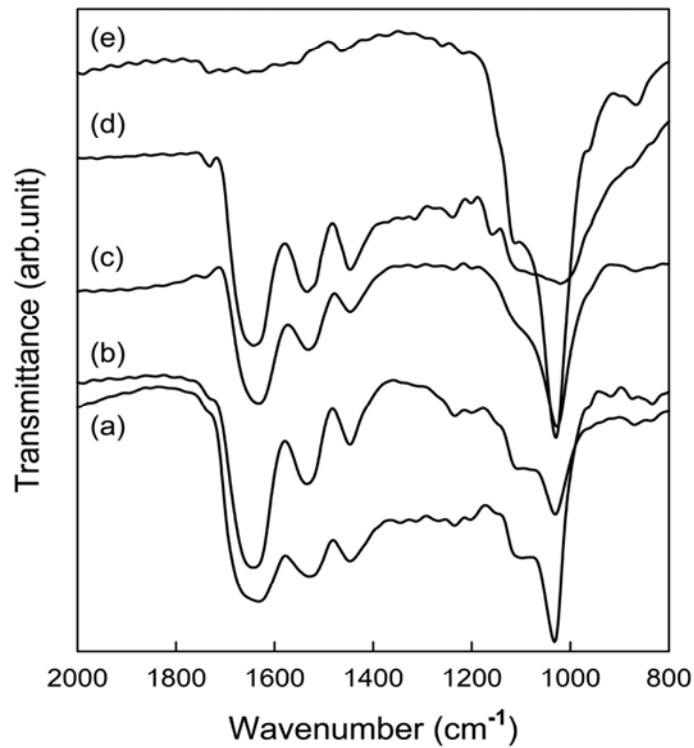


Fig. S1: FT-IR spectra of calcium phosphate minerals produced in the presence of rP147 (a), rP172 (b), P148 (c), P173 (d) and absence of protein (e) as a control.

FT-IR analyses of mineral phases formed in the presence of porcine amelogenins. FT-IR spectroscopy of samples collected at 1 d was carried out to determine the nature of mineral phases formed under high ionic strength conditions (Fig. S1), using a Perkin-Elmer FT-IR Microscope in reflectance mode. Routinely, 128 of accumulated scans and 4 cm⁻¹ resolution were used. In the absence of protein (control)

(e), spectra were found to correspond to an a poorly crystalline apatitic phase, with a major absorbance peak at about 1035 cm^{-1} and a shoulder at 1102 cm^{-1} arising from asymmetrical ν_3 stretching vibrations of PO_4^{3-} [1]. The fact that these bands are not well enough resolved to reveal additional splitting, reflect the small size of the mineral particles formed and/or suggest that the resulting minerals have low crystallinity. In the presence of rP147 (a) and rP172 (b), phosphate peaks (1111 cm^{-1} and 1030 cm^{-1}) were observed indicating the formation of a crystalline HA phase. Interestingly, similar apatitic phosphate peaks (1111 cm^{-1} and 1030 cm^{-1}) were also observed for samples prepared in the presence of P148 (c) unlike the FT-IR spectrum of minerals obtained under low ionic strength conditions, as shown in previous research [2]. In contrast, in samples prepared in the presence of P173 (d), a broad, featureless band was observed at ~ 900 to 1200 cm^{-1} , in the ν_3 phosphate region at ~ 900 to 1150 cm^{-1} , characteristic of ACP [1], as shown in Fig. S4. In all samples containing the porcine amelogenins studied, Amide I and II bands in the 1700 - 1600 cm^{-1} and 1600 - 1500 cm^{-1} region, respectively, were observed, indicating the presence of proteins in the reaction products. The band between 1400 and 1500 cm^{-1} represents protein side-chain vibrations. Amide III is usually seen between 1300 and 1200 cm^{-1} . Thus our FTIR analyses of the mineralization reaction products are consistent with SAED results obtained for 1 d control samples and samples prepared in the presence of rP172, rP147, P148 and P173 presented in Figs. 3-5. As noted in the text, due to the extremely small amounts of mineral formed in our mineralization system, the FT-IR spectra of mineral products obtained in this fashion became extremely noisy below 600 cm^{-1} . Thus, absorption bands in this region could not be used to further aid in mineral phase characterization.

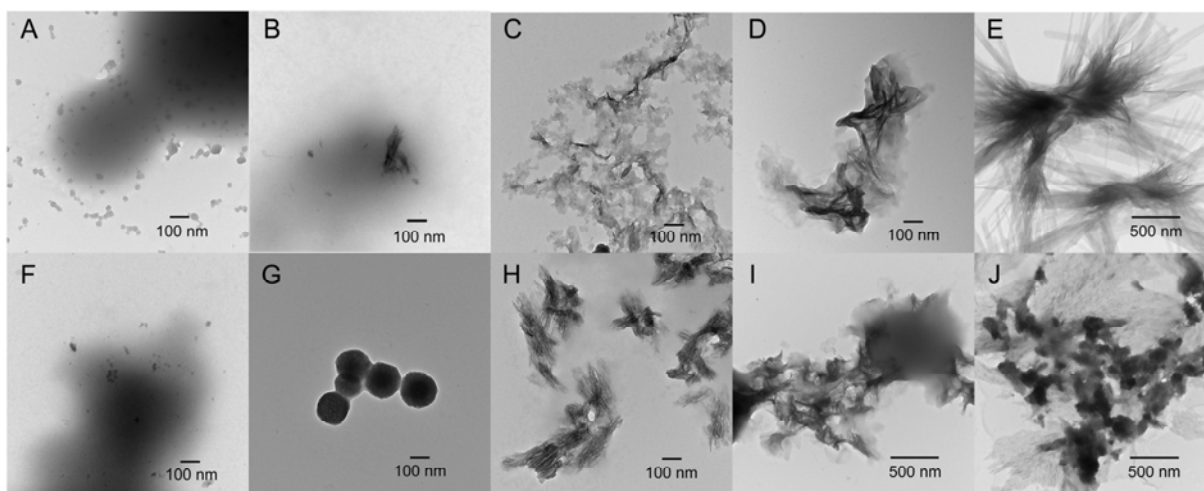


Fig. S2: TEM images of minerals formed in the presence of P148 under conditions of high ionic strength at 24 h (upper row A-E) and 48 h (lower row F-J).

Additional TEM images of minerals formed during mineralization experiments under high ionic strength conditions. As shown in Fig. S2, and discussed in the text, mineral formed in the presence of P148 at 24 h (A~E) and 48 h (F~J) varied greatly with respect to size and orientation.

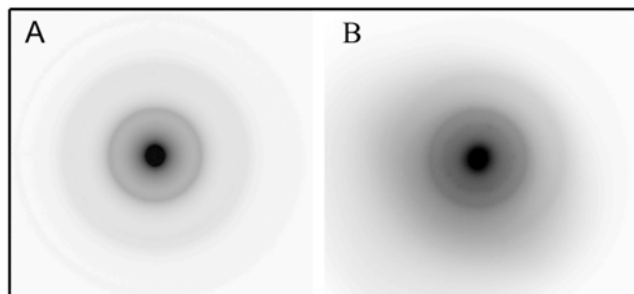


Fig. S3: SAED patterns of carbon-coated Cu grid (A) and synthetic ACP (B).

SAED analyses of synthetic ACP. To further confirm our characterization of ACP, SAED analyses of synthetic ACP were carried out in the same manner as used to characterize products obtained from our mineralization system. As shown in Fig. S3B, the SAED pattern observed for synthetic ACP is essentially identical to those shown in Figs. 3A, 4A, C and 5A, C, D, verifying the amorphous nature of these initially form mineral particles, as described in the text. We have also included an SAED pattern of a carbon-coated grid (Fig. S3A) alone to account for its contributions to the observed amorphous pattern, as we have previously reported [3].

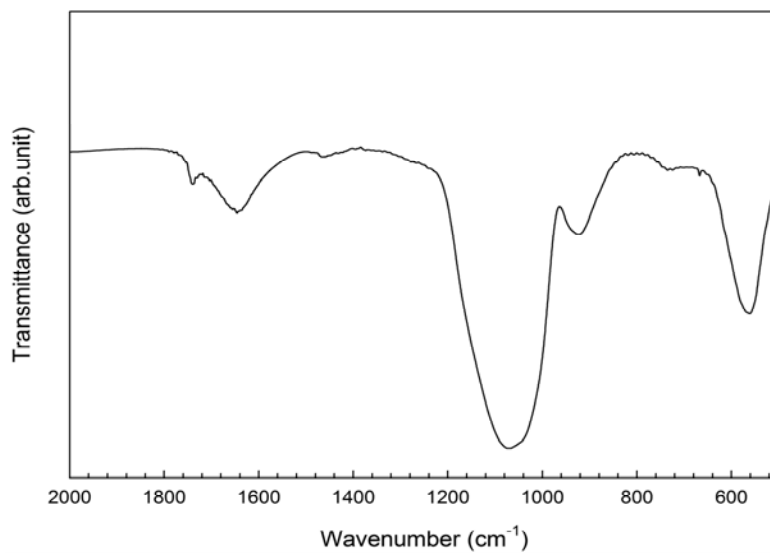


Fig. S4: Conventional FT-IR spectrum of synthetic ACP.

Stabilized ACP was prepared by using a modification of a previously described procedure [3], with the addition of a small quantity of pyrophosphate. Stabilized ACP was prepared by mixing 100 mM CaCl₂ and 100 mM Na₂PO₄ with Na₄P₂O₇ (final concentration: 1.2 μM) in distilled water to final concentrations of 5 mM and 3 mM, respectively, under ambient conditions with stirring. The resulting pH was ~7.1. Thirty minutes after the beginning of the reaction the mineral suspension was centrifuged at 8000 × g at 20°C for 5 min. The precipitate was then lyophilized at -20°C. The prepared ACP was characterized using conventional FT-IR and was found to exhibit characteristic broad ν₄ and ν₃ phosphate bands at around 565 cm⁻¹ and 1056 cm⁻¹, as shown in Fig. S4, consistent with other reports [1, 3].

1. GADALETA SJ, PASCHALIS EP, BETTS F, MENDELSON R, BOSKEY AL. Fourier transform infrared spectroscopy of the solution-mediated conversion of amorphous calcium phosphate to hydroxyapatite: new correlations between X-ray diffraction and infrared data. *Calcif Tiss Int* 1996; **58**: 9-16.
2. KWAK SY, WIEDEMANN-BIDLACK FB, BENIASH E, YAMAKOSHI Y, SIMMER JP, LITMAN A, MARGOLIS HC. Role of 20 kDa amelogenin (P148) phosphorylation in calcium phosphate formation in vitro. *J Biol Chem* 2009; **284**: 18972-18979.
3. BENIASH E, METZLER RA, LAM RSK, GILBERT PUPA. Transient amorphous calcium phosphate in forming enamel. *J Struct Biol* 2009; **166**: 133-143.