Crystallization of Calcium Phosphate Templated by α-Amino Acids Depending on Their Composition, Chain Length, and Enantiomerism

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α-Amino acids showed a characteristic templating effect on the crystallization of calcium phosphate at pH 7.0. Neutral but polar or basic group bearing amino acids yielded hydroxyapatite predominantly, whereas *n*-alkyl amino acids produced octacalcium phosphate (OCP) leading to an increase in crystallinity, expansion in cell volume and/or interlayer contraction depending on their chain length and enantiomerism. Aspartic acid, a short chain acidic amino acid, formed an amino acid complexed OCP with a significant increase of its crystallinity in the order of D ≈ L < DL, in contrast to the partial formation of a similar composite for glutamic acid.

Increasing attention has been paid to the mineralization in living systems, because of its biological and biomedical importance as well as the applicability of the biological strategies inherent in the mineralization to chemical technology.[1–4] In the calcification into bone or tooth the templating complexation of apatite with collagen is important as the essential process. Kniep and Bush, for example, demonstrated the biomimetic growth and self-assembly of fluoroapatite aggregates by diffusion into denatured collagen matrices.[5] In the calcification process initiated in osteoblast, octacalcium phosphate (OCP; $Ca_8(PO_4)_4(HPO_4)_2{\cdot}5H_2O$)[6] has been believed to serve as the precursor crystal to transform topotactically into apatite[7] or induce the epitaxial growth of apatite.[8,9] It is therefore of great interest to clarify the role of such non-apatitic calcium phosphates or other biospecies in calcification processes. Here, we report the preferred crystallization of either hydroxyapatite (HAp), OCP or its composite through a templating effect of different types of DL-, D- and/or L-α-amino acids depending on their electrostatic, chain length, and enantiomeric characters.

Reagent grade $Ca(NO_3)_2{\cdot}H_2O$, KH_2PO_4, amino acid, urea, and water were mixed at a molar ratio of 1:0.6:3:30:592 and adjusted to pH 3.0 with 1 mol dm^{-3} HNO_3. After the mixture being heated at 70 °C for 24–57 h to reach to pH 7.0, the resulting solid was separated, fully washed with water, and dried in air.

In the amino acid free system, the solid, **1**, separated at pH 6.0 was a single phase of OCP, whereas the pH 7.0-product, **2**, was a mixture of OCP and HAp (Figure 1). This is because the OCP structure composed of alternately stacked apatite- and brushite-like layers[6] becomes less stable in alkaline or neutral solution. An HAp-more enriched mixture was produced when any of neutral but polar group bearing amino acid L-glutamine (Gln) and basic amino acids L-ornithine (Orn) and L-lysine (Lys) was added, as exemplified for the L-Gln-added product, L-**3**. On addition of DL-*n*-alkyl α-amino acid $C_nH_{2n+1}CH(NH_2)COOH$ (n = 0, 2, 3, 4), the resulting solids, **4**(n), increased remarkably in both crystallinity and OCP yield with an increase of n until the HAp phase disappeared for n ≥ 3 (Figure 1). The XRD peaks observed for each major phase in **4**(n) were indexed on the basis of a triclinic cell with the lattice parameters close to those reported in literature.[10] The parameters *a*, *b*, and *c* were little dependent on the carbon number n, while the unit cell volume increased slightly with an increase of n (Figure 2). The Ca/P molar ratios of 1.35 for **4**(3) and 1.30 for **4**(4), determined by EDX analysis, agreed closely with an ideal value of 1.33 for OCP. These observations suggest a templating effect of amino acid molecules on the growth of OCP crystal,

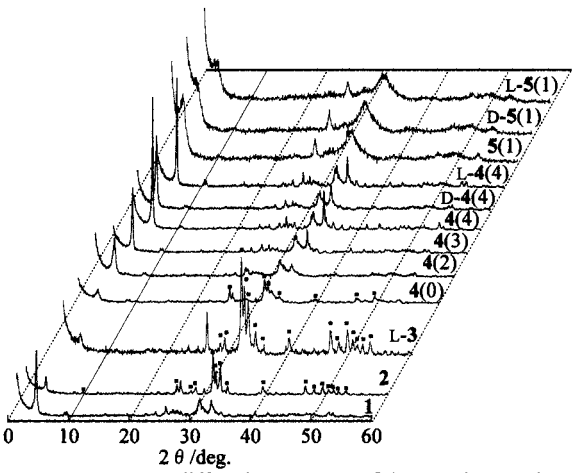

Figure 1. X-Ray diffraction patterns of the reaction products obtained with or without D-,L- and/or racemic α-amino acids. The peaks indicated by closed squares refer to hydroxyapatite.

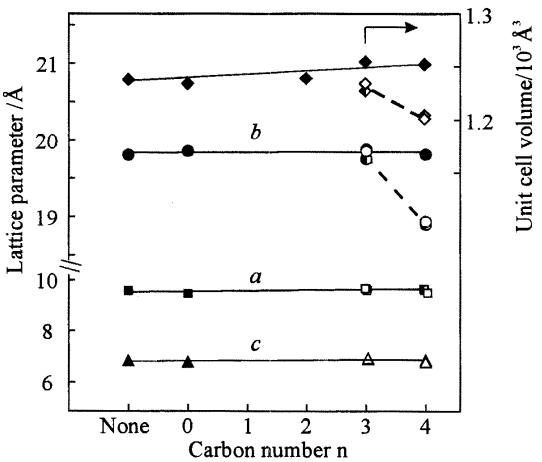

Figure 2. Plots of triclinic lattice parameters *a* (square), *b* (circle) and *c* (triangle) and unit cell volume (diamond) as a funkction of carbon number n for the products **2**, **3**(n) (closed), D-**3**(n) (open) and L-**3**(n) (half-closed).

although no trace amount of amino acid was detected for any **4**(n). More interestingly, D- and L-enantiomers with n = 4 showed much more marked templating effect, leading to amino acid free triclinic crystals, D- and L-**4**(4), with nearly the same *a* and *c* values as those for **4**(4), but with great decreases of 0.9 Å in *b* or 0.8 Å in interlayer spacing and 50 Å^3 in unit cell volume (Figures 1 and 2). A similar but much less pronounced effect was observed for the n = 3 enantiomer-added systems (Figure 2). The Ca/P molar ratio decreased slightly from 1.30 for **4**(4) to 1.24 for D-**4**(4) and 1.26 for L-**4**(4), suggesting a partial loss of Ca atoms in the OCP structure.

The amino acid templating effect suggested above might be attributed to the weak binding of amino acid molecules through their carboxyl and amino groups to the calcium and phosphate sites of surface brushite-like layers of growing OCP crystals. The relatively high concentration of amino acid used would also be effective for such molecular immobilization. Contrary to the hydrophilic group bearing Gln, Orn and Lys systems, the hydrophobic surface modified with the alkyl chain groups would serve to suppress the dissolution of the brushite-like layer leading to the subsequent layer-by-layer crystal growth. The crystal growth by such a mode would be more promoted with long chain and racemic amino acid molecules through the enhanced intermolecular interaction. In contrast, long chain but enantiomeric amino acid molecules would be less available for their close packing on the crystal surface. This may cause a calcium deficient structure, as observed for D- and L-**4**(4).

D-, L- and DL-Aspartic acids (Asp), acidic amino acids HOOC$(CH_2)_m$CH (NH_2)COOH with m = 1, formed a phase, D-, L- and DL-**5**(1), characterized by the OCP-like XRD pattern but with a peak or shoulder corresponding to a basal spacing of ca. 21.4 Å (Figure 1). This indicates the formation of an Asp complexed OCP expanded in interlayer spacing by 2.5 Å relative to 18.9 Å for OCP, with a marked dependence of its crystallinity on the enantiomeric character. The existence of amino acid species in these products was confirmed by their FT-IR spectra. Consistent with the assumption that they are a single phase with the composition of $Ca_8(HPO_4)_{2-x}(PO_4)_4(Asp)_y{\cdot}zH_2O$, amino acid analysis by the ninhydrin method coupled with the EDX and TG analysis gave close agreement between x and y in each product; x = 0.240, y = 0.254 and z = 9.5 for D-**5**(1), x = 0.244, y = 0.260 and z = 8.5 for L-**5**(1), and x = 0.281, y = 0.289 and z = 9.3 for DL-**5**(1). These x and y values also reveal that approximately one Asp molecule as a divalent anion per two unit cells replaces the equal number of HPO_4^{2-} anion in the OCP framework. We can thus propose a model in which the Asp molecules are placed with their two carboxyl termini ionically bonded to the calcium sites on the internal surface of the expanded channel along the *c* axis, as shown in Figure 3. The total volume of the incorporated Asp and water molecules, each 105 and 15 Å^3 in van der Waals volume,[11] per unit cell was calculated to be 339, 310 and 340 Å^3 for D-, L- and DL-**5**(1), respectively. These values are in close agreement with the sum of ca. 208 Å^3 for the cell volume increase relative to OCP and 150 Å^3 for the volume occupied by ten water molecules in it. The interlayer spacing of 21.4 Å for the Asp complexes also agrees with 21.5 Å for the same carbon number succinate complexed OCP formed via the hydrolysis of α-tricalcium bis(phosphate).[12] The Asp content in the formers, however, is as little as roughly one-third of the succinate in the latter. This depressive Asp/HPO_4^{2-} replacement is probably because the incorporated

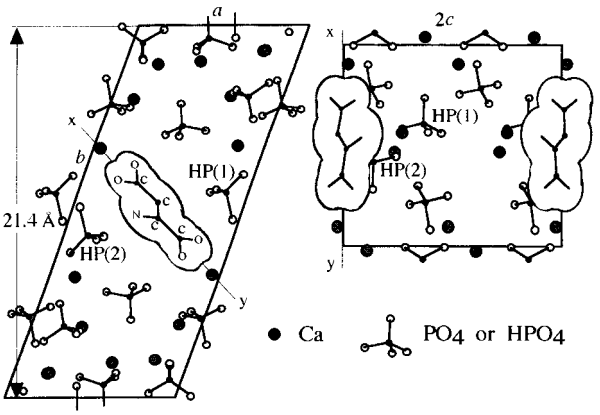

Figure 3. Model proposed for the arrangement of DL-aspartic acid molecules incorporated in the expanded channel of OCP framework.

Asp molecules are additionally hydrogen-bonded to any remaining HPO_4^{2-} group through their amino group with the N–H···O distance of ca. 3 Å, as observed for arginine phosphate.[13] This additional effect might favor predominant binding of L- or D-Asp molecule to either of the HPO_4^{2-} groups HP(1) and HP(2) (Figure 3). It would be more preferable, however, that alternate one of these HPO_4^{2-} groups is missed and that the trans zigzag plane of Asp molecule is oriented as parallel as possible to the *c* axis. This may lead to the particularly interesting tendency of D ≈ L < DL in both crystallinity and Asp content for the present complexes. In striking contrast to the m = 1 system, D-, L- and DL-glutamic acids (Glu, m = 2) yielded a slight amount of Glu-complexed OCP mixed with OCP as the major phase.

The present observation is the first example for the electrostatic, enantiomeric and chain length effects of amino acids on the crystallization of calcium phosphate. New knowledge for stereospecific properties of organic or inorganic implicit species in biomineralization may also be useful for the design of apatite–collagen composite materials.[14,15]

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