Crystallization in Aggregates of Calcium Phosphate Nanocrystals: A Logistic Model for Kinetics of Fractal Structure Development

Sha Bian,† Lin-Wei Du,† Yu-Xi Gao,‡ Jian Huang,† Bao-Di Gou,*† Xiuhong Li,§ Yi Liu,§ Tian-Lan Zhang,*† and Kui Wang†

†Department of Chemical Biology, Peking University School of Pharmaceutical Sciences, 38 Xueyuan Road, Beijing 100191, P.R. China
‡Key Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Academy of Sciences, 19B Yuquan Road, Beijing 100049, P.R. China
§Shanghai Synchrotron Radiation Facility, Shanghai Institute of Applied Physics, Chinese Academy of Science, 239 Zhang-Heng Road, Shanghai 201204, P.R. China

ABSTRACT: Calcium phosphate crystallization is of importance in medicine and materials science, and aggregation frequently happens at its early stage. Herein, we report a kinetic study of crystallization in particles of aggregated nanocrystals of calcium phosphate by in situ synchrotron small-angle X-ray scattering. The particles were random fractal aggregates, having diameters of 20–60 nm and comprising structural units of 2.5 nm in dimension. With the application of a logistic model, the structural changes of the particles were characterized by the successive developments of mass and surface fractal dimensions. The rate constant of mass fractal development was found to be 0.052 h⁻¹, corresponding to the generation of crystallites on the pre-existing seeds inside an aggregate. These newly formed nanocrystals then detached from their seeds and fell into the voids. Afterward, the surface fractal structure developed at a rate of 0.11 h⁻¹, making the particle surface less rough. During this period, a transition layer of 1.2-nm thickness emerged between a particle and the surrounding solution. Our findings present an advance toward understanding the crystallization in particles of aggregated nanocrystals and would be beneficial for preparing nanocrystals and recognizing the roles of other substances (such as proteins and metals) in both biological and synthetic systems.

INTRODUCTION

Crystalization of calcium phosphate is an important issue in medicine¹ and materials science.² Among all of the mineral phases of calcium phosphate, hydroxyapatite [HAP, Ca₁₀(PO₄)₆(OH)₂] is the least soluble in alkaline and neutral solutions. However, HAP seldom precipitates directly from a supersaturated solution, but rather forms through the conversion of some metastable precursors such as amorphous calcium phosphate (ACP), dicalcium phosphate dihydrate (DCPD, CaHPO₄·2H₂O), and octacalcium phosphate (OCP, Ca₈H₂(PO₄)₆·5H₂O).³ The conversion process is susceptible to the influence of various substances, such as proteins, synthetic polyelectrolytes, and endogenous and exogenous metal ions. Although the roles of these substances in crystallization are currently the subject of numerous investigations, the mechanism of calcium phosphate crystallization on the nanometric scale, even in the absence of any additive, has not yet been fully elucidated. Therefore, a further understanding of the basic process would be beneficial for recognizing the roles of various additives in calcium phosphate crystallization in both biological and synthetic systems.

Aggregation frequently takes place with the as-formed nanocrystals of calcium phosphates at the early stages of crystallization, and the subsequent structural changes in particles of aggregated nanocrystals are difficult to characterize in situ by conventional experimental techniques. The tendency to aggregate also presents one of the key issues in molecular simulations.⁴ However, the feature of self-similarity with aggregates can be employed in characterizing their structure. Aggregation, either between primary particles or between their aggregates, results from Brownian motion, which generates self-similar structures in these aggregates.⁵ Accordingly, one can apply the concept of fractal geometry to the description of the self-similar structure and derive such information from scattering measurements.⁶–⁷ An example is the characterization of a protein-mediated structural change in calcium phosphate particles.⁸ Most recently, a mass-surface fractal transition was reported in the formation of silver nanocrystals.⁹ In the study of crystallization kinetics by scattering techniques, however, the most frequently determined quantities are crystal size,¹⁰ number,¹¹ and total volume.¹² We have previously reported the formation of HAP nanocrystals inside an ACP particle.¹³ The HAP nanocrystals, as soon as they were released from the ACP particles, would
aggregate because of their large specific surface areas. The aim of the present study was to investigate the kinetics of calcium phosphate crystallization in particles of aggregated nanocrystals by the in situ synchrotron small-angle X-ray scattering (SAXS) technique. With the excellent light source from synchrotron radiation, the measurements were carried out with a greatly elevated signal-to-noise ratio, making it possible to monitor progressively the structural changes in such particles. In particular, by setting the appropriate sample-to-detector distance (SDD), we managed to choose the “observation window” of 1–70 nm and focus on just one of the hierarchical structures of the particles, without being seriously impeded by others. This strategy might add one more advantage to those mentioned in a recent review of SAXS.14 Conventional X-ray diffraction (XRD) and transmission electron microscopy (TEM), although not suitable for characterizing small crystals of a few nanometers in the presence of larger ones, were used to confirm the coexisting solids, among them the precursor phases (ACP, OCP, and DCPD) providing the lattice ions for HAP crystal growth. The calcium and proton concentrations reflected the progress of crystallization both inside and outside the particles of aggregated nanocrystals, and they acted as a complement to the physical information from SAXS measurements, providing clues to the mechanism of phase transformation.

■ EXPERIMENTAL SECTION

Materials. CaCl₂·2H₂O, NaH₂PO₄, HAP, and ACP used in XRD measurements were obtained from Sigma. The standard solution for calcium calibration was provided by Thermo Scientific. Other chemical reagents were of analytical grade. Water was deionized and doubly distilled before use. Calcium and phosphate solutions were prepared fresh, adjusted to pH 7.40 at 25 °C, and filtered through 200-nm pore-size filters (PES Membrane, Jinteng) by a vacuum-filtration system.

SAXS Measurement. We followed the course of crystallization using the in situ synchrotron SAXS technique. All measurements were performed at ambient temperature (22 °C) with a three-slit collimation system at beamline BL16B1 of the Shanghai Synchrotron Radiation Facility (SSRF), Shanghai, China. The calcium phosphate suspension was prepared by mixing 15 mL of CaCl₂ (80 mmol·L⁻¹), pH 7.40 and 15 mL of sodium phosphate (48 mmol·L⁻¹), pH 7.40. Precipitation immediately occurred, making the pH of the suspension fall to 5.80. Because acidic medium could retard the phase transformation, we reduced the acidity of the suspension by removing 20 mL of supernatant after centrifugation (Anke GL-20B) at 4000 g for 2 min and then adding 20 mL of phosphate buffer (4.8 mmol·L⁻¹, pH 7.60). After repeating this procedure once and removing the supernatant (to raise the signal-to-noise ratio in SAXS measurement), we loaded the suspension (pH 6.45, 0.70 mL) into 2-mm-thick sample cells, whose front and back windows were both covered with Kapton tape. The sample cells were then mounted onto a holder in the beamline at a sample-to-detector distance (SDD) of 1820 mm. Additional SAXS experiments were carried out at SDDs of 1830, 2999, and 5182 mm. The area of the primary X-ray beam at the sample position was 0.4 × 0.5 mm² with a wavelength of 0.124 nm. At SDD = 1820 mm, the range of effective scattering vectors q is 0.11–2.16 nm⁻¹, where q = (4π sin θ)/λ and 2θ is the scattering angle. To exclude the possible effects of irradiation on the crystallization, we measured each sample once only and then discarded it. We recorded 2D SAXS patterns with a Mar165 charge-coupled device (CCD) detector and obtained scattering curves by converting the original data with the software Fit2D. By measuring sample adsorption using ionization chambers in front and back of the sample cell, we performed data correction, calibrated the SAXS data for background scattering, and normalized the data with respect to the primary beam intensity.

Calcium Concentration and pH Measurements. We followed the changes in calcium concentration and pH in the suspension during the crystallization process. Aliquots of a calcium phosphate suspension, prepared by the same procedure as used for SAXS measurements, were placed in plastic centrifugation tubes. At various time intervals, we withdrew 5–10 mL of supernatant from the upper part of the suspension and measured the pH or calcium concentration using a pH meter coupled with an ionic-selective electrode (Mettler Toledo LE438 for H⁺ and Thermo-Orion 97–20 Ionplus for Ca²⁺). After recording the value of the remained slurry volume in the plastic tube, we returned the supernatant and measured the pH or calcium concentration again. Let V and c be the volume and concentration, respectively, of protons or calcium ions. The proton or calcium concentration in the slurry was calculated according to the equation c₂ = (c₁V₁ − c₂V₂)/V₂, where the subscripts 1, 2, and t represent supernatant, slurry, and total, respectively.

XRD and TEM Measurements. We examined the solid phases by XRD and TEM. Calcium phosphate suspension was prepared by the same procedure as used for the SAXS measurements. At each settled time, the water in a sample was removed by filtration through a 200-nm pore filter (PES Membrane, Tianjin Jinteng). After successively washing the slurry with 40 mL of anhydrous ethanol twice and with 40 mL of acetone three times, we removed the solvent as completely as possible by passing the suspension through a 200-nm pore filter (Nylon 66 Membrane, Tianjin Jinteng) with a vacuum-filtration system and then kept the solid in a desiccator under reduced pressure for 1 h at ambient temperature. The solid was then sealed in a 1.5 mL Eppendorf tube for XRD and TEM analyses.

The XRD measurements were conducted on a Rigaku D/Max-RB instrument using Cu Kα radiation at a scanning speed of 7° 2θ min⁻¹.

Figure 1. XRD patterns of calcium phosphate solids. (a) Samples isolated from the suspension at various times. The letters O, B, and H indicate peaks of OCP, DCPD (brushite), and HAP, respectively. (b) Pure HAP, pure ACP, and their mixture (50:50 w/w).
The operating voltage and current were 40 kV and 150 mA, respectively. Phase identification was performed with the XRD analytical software Jade 5.0 (MDI, Livermore, CA) using the powder diffraction file (PDF). PDF card 09-0077 was used as the reference for DCPD, card 09-0432 for HAP, and card 26-1056 for OCP. TEM measurements were conducted on a Hitachi H-9000 NAR instrument at an accelerating voltage of 100 kV to characterize the morphologies of the calcium phosphate solids.

**RESULTS AND DISCUSSION**

Several calcium phosphate phases coexisted in the system under study. As revealed in a previous report, ion pairs and clusters form immediately upon mixing calcium and phosphate solutions, each at near-neutral pH, and they further develop into ACP particles; inside these particles, HAP nanocrystals gradually form, causing the particles to disintegrate and triggering rapid crystallization in the supersaturated solution. In the initial suspensions for SAXS measurement, the crystallization-generated protons reduced the solution pH to 5.80. To facilitate the subsequent phase transformation, we washed these precipitates twice with phosphate buffer (4.8 mmol·L⁻¹, pH 7.60), elevating the solution pH to 6.45. Because most of the crystals formed at the initial pH of ~7.0, the dominant crystalline phases were OCP and HAP, whereas DCPD was a minor product (Figures 1a and 2a,b). The amount of ACP is hard to estimate, because of its much weaker diffraction intensity compared to that of crystals (Figure 1b). Among the solid phases in the suspension, the small HAP crystals coaggregated with ACP debris, forming particles with fractal features. The events that happened to these particles afterward are the main topic of the article.

In this study, we used XRD and TEM data with special caution for the following reasons: First, the sample preparation procedure could cause alterations in the samples. Second, the very small size and low crystallinity of the crystals in aggregates could lead to an underestimation of the small crystals in the presence of larger crystals. Third, the irradiation of high-energy electrons could cause DCPD-to-OCP and OCP-to-HAP transformations. Finally, the polycrystalline nature of the
TEM images could indicate larger dimensions than those derived from XRD data. Actually, the XRD and TEM data were limited simply to demonstrating the coexistence of various mineral phases, whereas the in situ synchrotron SAXS technique was used to follow the kinetics of structural changes in the particles of aggregated nanocrystals.

We display the SAXS patterns collected at various times as log($J$) versus log($q$) plots in Figure 3a and as $Jq^3$ versus $q^2$ plots in Figure 3b, where $J$ and $q$ are the scattering intensity and the modulus of the scattering vector, respectively. Each curve in Figure 3a shows a linear region in the range of $q = 0.12$–0.8 nm$^{-1}$. Scattering curves of this type have been interpreted as being indicative of fractal particles comprising structural subunits.

For the SAXS data collected with the slit collimation system, we define the fractal parameter $\alpha \equiv -d + 1$, whose value can be determined from the slope ($d$) of a linear region according to eq 1. The fractal parameter $\alpha$ is related to the mass fractal dimensionality ($D_m$) and the surface fractal dimensionality ($D_s$) through eqs 2 and 3, respectively.

$$J = Cq^d \quad (C \text{ and } d \text{ are constants}) \quad (1)$$

$$D_m = \alpha \quad \text{where} \quad 1 < D_m \leq 3 \quad \text{if} \quad 1 < \alpha \leq 3 \quad (2)$$

$$D_s = 6 - \alpha \quad \text{where} \quad 2 \leq D_s < 3 \quad \text{if} \quad 3 < \alpha \leq 4 \quad (3)$$

The development of the fractal structure can be characterized by a logistic equation. Because the parameters of the equation provide physical insight into the process, it has been used to describe the increase in crystal size. In contrast, herein, we used the model to capture the kinetics of structural change in the scattering particles. Equation 4a shows the differential logistic equation.

$$(Cq^d)' = r(F(t))$$

On integration, it is expressed linearly as eq 4b, where $r$ (h$^{-1}$) is the reaction rate and $\alpha_0$ and $K$ are the initial value and upper limit, respectively, of the fractal.
parameter \( \alpha \). The theoretical value of \( K \) is 4, representing scattering particles that are uniform and compact in electron density in their interior and are sharp and smooth on the surface. We plot \( \ln[Ka/(K-\alpha)] \) versus time \( t \) according to eq 4b. Quite unexpectedly, the plot reveals two linear regions prior to the upper asymptote of the curve (Figure 3c), indicating that the fractal structure of the particles developed at two distinct rates during the first 16 h of crystallization.

\[
\frac{da}{dt} = ra(1 - \alpha/K) \quad (4a)
\]

\[
\ln\left(\frac{Ka}{K-\alpha}\right) = rt + \ln\left(\frac{K\alpha_0}{K-\alpha_0}\right) \quad (4b)
\]

Before moving on to the structural changes in the two stages, we first discuss the size and structure of the scattering particles. For homogeneous particles, the radius of gyration (\( R_g \)) is defined as the mean square distance from the center of gravity:

\[
R_g = \left(\frac{1}{n}\sum_{i=1}^{n} r_i^2\right)^{1/2} \quad (26)
\]

Each curve in Figure 3a exhibits a linear region in the range of \( q = 0.12-0.80 \text{ nm}^{-1} \). From the low-\( q \) and high-\( q \) limits of a linear region, one can estimate the value of \( R_g \) of the scattering particles and the average values of their structural units, respectively, using the relation \( R_g = 1/q^\alpha \).

By assuming the scattering particle to be a spherule and its structural units to be cubes, one can use these \( R_g \) values to estimate their sizes. The calculated diameter of a particle is \( 2 \times 1.29/0.12 = 21.5 \text{ nm} \), and the side length of the cubes is \( 2/0.8 = 2.5 \text{ nm} \). For a polydisperse system, because one expects \( q_{\text{min}}R \gg 1 \) when power-law scattering is observed, the average dimension of the particles can be conservatively estimated by \( R = 3.5/\gamma_{\text{min}} = 29 \text{ nm} \), that is, diameter = 58 nm. Taking the average of 21.5 and 58 nm, we tentatively describe the scattering particles as spherules that have diameters of about 40 nm and comprise cubic structural units with dimensions of 2.5 nm. The particle size estimated from the low-\( q \) limit of a linear region is not restricted by the truncation effect at SDD = 1820 nm, a judgment that was confirmed in an additional SAXS experiment at SDD = 5182 nm. There exist two linear regions in the lowest curve presented as a broad, black line in Figure 3a. While the right one corresponds to the linear region observed at SDD = 1820 nm, the left one indicates a higher order in the structural hierarchy of the scattering particles. We focus on the data obtained at SDD = 1820 mm in the subsequent discussion.

The scattering particles are the aggregates of HAP nanocrystals, likely containing ACP. The coexistence of DCPD, OCP, and HAP is indicated in XRD (Figure 1a) and TEM (Figure 2a,b) data at 0.5 h. The bump around 2\( \theta = 28^\circ \) and the higher background at 2\( \theta = 20-30^\circ \) indicate the presence of nanocrystalline HAP and ACP, respectively. In such a system containing several mineral phases, the supernatant is just saturated with respect to the most soluble one and supersaturated with respect to the others. Therefore, as soon as the crystallization of a less-soluble mineral phase proceeds, the supernatant becomes undersaturated with respect to the most soluble one and the latter dissolves. Because the solution pH ranged from 6.4 to 5.8 (Figure 4a), the first dissolved solid was ACP, followed by OCP and/or DCPD. Accordingly, neither of the two crystalline phases matched the constantly developing feature of the scattering particles. Therefore, the least-soluble HAP must account for the monotonic progress of the fractal structure throughout almost the whole observation period. Previously, we observed 5-nm HAP crystallites that developed inside ACP particles and were released into solution afterward. In the present study, the initial concentrations of calcium and phosphate were 10-fold higher and, therefore, would yield more ACP particles. As a direct consequence of the increased collision frequency, these particles would collapse sooner and release smaller crystallites (2.5 nm, as derived from the SAXS data in Figure 3a). Hence, the most feasible candidate for the scattering particles would be HAP nanocrystal aggregates containing the debris of broken ACP particles. Formed under highly nonequilibrium condition, such aggregates are typically fractal in nature. The incipient fractal dimensionality of the scattering particles was 2.79 at 0.5 h (where \( D_m = \alpha \)), close to that (\( D_m = 2.5 \)) of the particles formed through diffusion-controlled monomer–cluster aggregation.

Now, we analyze the structural changes in the particles of aggregated HAP nanocrystals. The first stage is indicated by the left linear region in Figure 3c (0.5–6.5 h). During this period, the fractal parameter \( \alpha \) increased from 2.79 to 3.05 at a rate of \( r = 0.052 \text{ h}^{-1} \), corresponding to a change toward more compact and more uniform particles in terms of electron density. Such a change can also be seen from the reduction of the positive deviation from Porod’s power law in Figure 3b. To account for...
for the mass fractal development, crystallization must have taken place in the interior of these particles, although crystallization on the surface cannot be excluded. The nanocrystals inside a particle could act as seeds, and the seeded crystallization could lead to two possible consequences: (1) the pre-existing crystals grow, each remaining a single crystal, and (2) new crystals form on the seeds’ surface and then detach, falling into the voids inside the particle. Further examination of the curves in Figure 3a might help to indicate the case that actually occurred. There is another linear region between $q = 0.8–2.0 \text{ nm}^{-1}$, in addition to that between 0.12–0.8 nm$^{-1}$. The extension lines of the two linear regions in the curve intersect, and all of the intersection points of the scattering curves fall around $q = 0.8 \text{ nm}^{-1}$, implying a roughly fixed size of the structural units during this period. Hence, the development of a mass fractal must have resulted from the second consequence, that is, the generation of more crystallites rather than the enlargement of existing seeds. This feature of crystallization, although quite unexpected, might be experimental evidence for the prediction of a simulation study: The seeds act as crystallization “catalysts” if they are just above a well-defined minimum size, and newly formed crystallites detach from these seeds. Moreover, the acidic environment inside the particle, as exhibited in Figure 4a, might also impede the growth of the pre-existing seeds. However, the preformed ion clusters, in addition to the seeds, might facilitate the formation of new crystallites, thus making it difficult to detect crystallites of intermediate sizes. Indeed, in this stage, we did not observe a marked change in the short-range correlation, as was indicated by the slope of the linear region in the high-$q$ range. At the end of the first stage (6.5 h), the fractal dimensionality ($D_L = 2.95$) corresponded to particles that are uniform and dense in their interior but very rough on the surface. The rough surface might be a consequence of ACP dissolution by the crystallization-generated protons. Both inside and outside the particles of aggregated nanocrystals, the dissolution of ACP counteracted, at least in part, the increase in proton concentration in this stage (Figure 4a).

The second linear region in Figure 3c (6.5–16 h) represents a stage that is characterized by the development of a surface fractal. This stage is different from the previous one in several aspects. First, the structural changes took place mainly on the particle surface. The development of the surface fractal yielded a higher value of $D_L$, leading to a lower $D_s$ value (eq 3) and suggesting a less rough surface. The rate ($r = 0.11 \text{ h}^{-1}$) is twice that of the mass fractal development. This difference in rate might reflect the different amounts of crystals in building a unit of the respective fractal structure. Moreover, the more facile dissolution of the crystallization-generated protons might also contribute to the higher rate on the particle surface. Second, the acidity of the suspension increased more rapidly (Figure 4a). At the beginning of the second stage (6.5 h), the slurry at the lower part of the suspension (pH 6.22) was slightly more acidic than the supernatant (pH 6.35). However, this difference increased at the end of the stage (16 h), with pH 5.92 for the slurry and pH 6.19 for the supernatant. Because ACP could not sustain this acidity, it must have completely dissolved earlier.

Hence, the crystal growth of HAP was at the expense of OCP and DCPD in proton generation during the phase conversion. The dominant form of phosphate is expressed as $\text{H}_2\text{PO}_4^-$ at about pH 6.0. Note the difference in HAP “molecule number” at the right side of the two equations. Finally, the calcium concentration was higher in the slurry than in the supernatant (Figure 4b), confirming that the lattice ions for HAP crystal growth were from the dissolution of its precursor(s) outside the scattering particles. Hence, dissolution-recrystallization was the way in which OCP transformed into HAP. Previously, a direct solid transformation through structural adjustment has been reported. The sudden changes between 11 and 15 h in both $\text{H}^+$ and Ca$^{2+}$ concentrations were well reproducible. These “anomalous” phenomena could be caused by the $\text{H}^+$-induced nonlinear change in the slurry density. The changes in proton and calcium concentrations (Figure 4) could affect the charges and diffusion rates of calcium and phosphates, as well as their ion pairs/clusters. Nevertheless, the rate of the surface fractal development remained constant throughout the stage, thus suggesting a reaction-controlled process. The same reasoning might also hold for the mass fractal development.

$$5\text{Ca}_4(\text{HPO}_4)_2(\text{PO}_4)_2\cdot5\text{H}_2\text{O}$$

$$= 4\text{Ca}_{10}(\text{PO}_4)\text{OH}_2 + 6\text{H}_2\text{PO}_4^- + 6\text{H}^+ + 17\text{H}_2\text{O}$$

(5)

$$10\text{CaHPO}_4\cdot2\text{H}_2\text{O} = \text{Ca}_{10}(\text{PO}_4)\text{OH}_2 + 4\text{H}_2\text{PO}_4^- + 4\text{H}^+ + 18\text{H}_2\text{O}$$

(6)

An additional event in this stage is the emergence of a transition layer between a particle and its surrounding solution. For the SAXS data collected with a slit collimation system, Porod’s power law predicts a flat final slope in the $q^2$ versus $q$ plot for idealized scattering particles; a positive deviation indicates fluctuations in electron density within a particle, whereas a negative one suggests the presence of a transition layer between a particle and its matrix. As exhibited in the high-$q$ region of the curves in Figure 3b, the deviations changed progressively from positive to negative, with a switching time at 11 h. According to eq 7, we determined the values of the transition layer thickness ($E$) from $q_j$ versus $1/q_j^2$ plots for the samples from 11 h on and display their temporal change in Figure 3d. “Transition layer” is a term in the field of SAXS, and it is frequently referred to as “surface layer” or “hydration layer” in crystallography. A hydration layer has previously been observed with HAP crystals and estimated to be thinner than 2 nm. It is reported that such a layer, which seems to exist in its nascent state only in wet samples and is altered upon drying, progressively disappears as the stable apatite domains develop. Recent NMR studies have demonstrated the existence of a hydrated, highly disordered surface layer of the crystallites in bone tissues.

Indeed, the composition and structure of a transition layer on a crystal surface, as well as its dynamic mass exchange with the surrounding matrix, might play important roles in crystallization/dissolution and deserve further examination.

Several factors might contribute to the emergence of the transition layer, as well as the development of the surface fractal. When newly formed crystals are sufficient on a particle surface, they can locate so closely that their hydration layers merge and connect with each other to form a layer enwrapping the whole particle. Moreover, the crystallization-generated...
protons dissipate into the surrounding solution more readily from the surface than from the interior of a particle. Consequently, the crystal growth on particle surface might reach a higher rate and produce larger sizes, as compared with the crystallization inside a particle. In addition, some nanocrystals on a particle’s surface might align with the same orientation and fuse into larger crystals or mesocrystals. However, because we did not observe any evidence for this in the SAXS patterns and because neither TEM nor XRD were efficient in charactering the particles of aggregated nanocrystals, the feasibility of the last factor remains to be demonstrated in further investigations.

\[ J_q = K_p/q^2 - CK_p \]

(where \( C \) and \( K_p \) are constants, \( C = E^2/6 \) )

In the last stage (16–24 h), the development of the surface fractal made little progress. When the structural changes terminated quite abruptly at the end of the second stage (16 h, Figure 3c), the fractal parameter \( \alpha \) was 3.60, not reaching its theoretical value of 4. This fact might represent a significant difference between chemical and physical crystallizations. In the latter, such as sucrose crystallization from aqueous solution, the solute molecule is the same as the molecule in the crystal, and the crystallization does not stop until the solute concentration constantly decreases to a certain level. However, in a chemical crystallization such as the case in the present study, the reaction stopped when the solution acidity reached \( \text{pH} \approx 6.0 \) at 16 h (Figure 3c), although the calcium and phosphate concentrations remained high. The accompanying proton release (eqs 5 and 6) could influence the crystallization in complex ways. On one hand, the higher concentration of protons made the coexisting OCP and DCPD more soluble (Figure 4, upper curves), providing more lattice ions for HAP crystal growth. On the other hand, it reduced the rate of HAP crystallization. The abrupt end of the progress of the surface fractal development seems to be indicative of a critical proton concentration (\( \text{pH} \approx 6.0 \)) for crystallization under the experimental conditions. One way the protons reduced the reaction rate might be associated with the transition layer. The crystallization of HAP requires the incorporation of deprotonated phosphate \( \text{PO}_4^{3-} \), dehydrated \( \text{Ca}^{2+} \) and \( \text{OH}^- \), as well as the release of hydrated protons and protonated phosphates (\( \text{H}_2\text{PO}_4^- \) and/or \( \text{HPO}_4^{2-} \)). During crystallization, the two types of substances pass through the transition layer in opposite directions. Accordingly, the efficiency of mass exchange must depend strongly on the composition and structure of the transition layer, and the thickness reflects the composition and structure of the transition layer. As is presented in Figure 3d, the thickness reached a maximum of 1.16 nm at 20 h and then dropped to 1.04 nm at 24 h. These changes were simultaneous with the slowing of the fractal structure development (Figure 3c). Although small in magnitude, the reduction of the transition layer thickness was well reproducible in an additional SAXS measurement at SDD = 2999 mm and thus should not be attributed to experimental errors. Considering the less compact nature of the transition layer compared to the enwrapped solid phase, the former must be more sensitive to various influences from the surrounding matrix. Therefore, the transition layer would not only be of importance in crystallization and dissolution, but also provide the locus where proteins and metal ions manifest their effects. An impact of the transition layer could be expected on bone mineral metabolism. Finally, we note that the structural changes indicated by the fractal dimension are restricted to the scattering particles, whereas the larger crystals revealed in XRD and TEM (Figures 1a and 2c,d) are beyond the scope of the SAXS measurements and might not necessarily behave in the same way.

In summary, we have followed the structural changes in particles of aggregated nanocrystals by the in situ synchrotron SAXS technique and, for the first time, analyzed the kinetics of fractal structure development using a logistic equation. The process is depicted schematically in Figure 5. The scattering particles were random fractal aggregates, having diameters in the range of 20–60 nm and comprising structural units that had dimensions of 2.5 nm. First, new crystallites formed on the pre-existing seeds and then detached into the voids inside the particle, generating more crystallites rather than enlarging the existing ones. In this way, crystallization built up the mass fractal dimension of a particle, making it more compact and uniform in electronic density. Then, the structural changes on the particle surface followed, which were faster than the previous ones and made the surface smoother. During this period, a transition layer emerged between a particle and the surrounding solution. Both mass and surface fractal developments were reaction-controlled processes. Finally, the structural changes of the particles hardly proceeded when the solution acidity fell to \( \text{pH} \approx 6.0 \), a factor that might obstruct crystallization by altering the thickness (as well as the composition and structure) of the transition layer.

**AUTHOR INFORMATION**

**Corresponding Author**

*Fax: 86-10-62015584. Tel.: 86-10-82801539. E-mail: tzhang@hsc.pku.edu.cn (T.-L.Z.), bgou@hsc.pku.edu.cn (B.-D.G.).

**Notes**

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