Amorphous 1-D nanowires of calcium phosphate/pyrophosphate: a demonstration of orientated growth of amorphous minerals

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Abstract
Amorphous inorganic solids are traditionally isotropic, thus, it is believed that they only grow into morphologies of nanospheres or irregular aggregates of nanoparticles. However, in the presence of (ortho)phosphate (Pi) and pyrophosphate ions (PPi) which have synergistic roles in biomineralization, amorphous calcium phosphate-pyrophosphate nanowires (ACPPNs) form in an aqueous additive-free solution. Cryo-TEM shows that the single nanowire has an average diameter of 2-3 nm, and lengths of up to hundreds of nanometers. In ACPPNs, amorphous calcium orthophosphate and amorphous calcium pyrophosphate are distributed at separated but close sites. The ACPPNs grow via either the preferential attachment of ~2 nm nanoclusters in 1-dimensional way, or the transformation of bigger nanoparticles. We propose that the anisotropy of ACPPNs microstructure, which is corroborated experimentally, causes their oriented growth. This study proves that, unlike the conventional view, amorphous minerals can form via oriented growth without external regulation, demonstrating a novel insights into the structures and growth behaviors of amorphous minerals.
Introduction

As an important inorganic mineral, calcium phosphate is abundant in nature and important for many organisms. For example, human bone is composed of 70% inorganic mineral (mainly apatite) and 30% organic matrix (mainly collagen), while tooth enamel is 97% hydroxyapatite, 1.5% proteins, and 1.5% water \[^1\]. Despite the diversity of the calcium phosphate family, each member has its role in tissue formation or biomedical applications \[^2\]. Among them, calcium orthophosphates (CaPi, where Pi stands for the orthophosphate ion) are attracting growing research interest. They include the amorphous form (amorphous calcium phosphate, ACaPi), and crystalline ones such as hydroxyapatite, octacalcium phosphate, brushite, monetite, and \((\alpha, \beta)\) tricalcium phosphates. Calcium pyrophosphate (CaPPI, where PPI stands for the pyrophosphate ion), also an important biomineral, has been much less discussed regardless of its crystalline or amorphous (ACaPPI) forms. CaPPI crystals are normally observed in pathological tissues, especially in cardiovascular and articular cartilage \[^3\]. Moreover, these ectopic calcifications are mostly composed of both CaPPI and CaPi crystals \[^4\]. In the process of bone formation, PPIs, which are mainly produced by hydrolyzing the phosphodiester bond in nucleotide triphosphates \[^5\], play an important role in regulating the formation of CaPi \[^6\], as they can effectively inhibit nucleation and crystallization of CaPi by antagonizing the binding of calcium with phosphate \[^6c, 6d, 7\]. It is believed that Pi and PPI synergistically control bone mineralization \[^6g, 8\].

During the formation of calcium phosphates from soluble ions to crystalline CaPi and CaPPI, amorphous phases (ACaPi and ACaPPI) evidently serve as a transition state, which also influences the structure and properties of the final minerals \[^9\]. The building units of ACaPi are considered to be Posner’s clusters with a size of \(\sim 9\ \text{Å} \[^{10}\), or CHPC clusters with a size of \(\sim 1-2\ \text{nm} \[^{11}\). However, again, despite various compositional studies, the microstructure of ACaPPI has been less studied, and no structural model has been proposed yet \[^{12}\]. Whatever the exact microstructures are, the amorphous solids, ACaPi and ACaPPI should have a certain short-range order, possibly even medium-range order, in the atomic arrangements \[^{13}\]. Like most amorphous solids, ACaPi and ACaPPI formed in aqueous environments without external regulation (i.e., templates, surfactants, electricity, etc.) show morphologies of round nanospheres or irregular nanoparticles \[^{14}\]. In comparison, their crystalline counterparts usually display morphologies with flat planes, distinct angles, sharp edges, and elongation in specific directions \[^{15}\]. Due to the energy differences between different facets of crystals, they prefer to grow in certain directions to minimize the total surface free energy according to Wulff’s rule \[^{16}\]. This thereby leads to corresponding facets exposed on the surface of crystals, forming distinct planes, angles, and edges, and also provides the possibility to modulate the size and shape of crystals \[^{17}\]. On the contrary, in amorphous solids, as the atoms and ions are ordered only within a very short range, they are isotropic when viewing them in the size scale of nanometers \[^{15c, 18}\), although their specific microstructures may give rise to anisotropy on very short length scale, that is, within the next-neighbors coordination shells. Therefore, there is usually no energetic driving force...
force for amorphous solids towards spatially preferential growth. Indeed, those conclusions are particularly applicable for the reported ACaPi and ACaPPI [18-19]. Hydroxyapatite nanospheres with thin amorphous layers were observed to assemble into needle-like crystallites, but the composition of the amorphous layer and its exact role during the formation of needles remained unclear [20].

However, in this study, we found an oriented growth behavior of an amorphous solid. Inspired by the fact that Pi and PPI co-exist in the bodily fluids and interact with each other, especially during biomineralization, we studied calcium phosphate formation in solutions including both of these species. Surprisingly, we obtained nanowires composed of amorphous solid with high aspect ratio in this study. The nanowire is composed of ACaPi and ACaPPI and its formation involves the assembly of nanoclusters or transformation of nanoparticles in an oriented fashion of 1-D. The amorphous nanowires thus provide a novel perspective on structures and growth behaviors of amorphous minerals.

Results and Discussion

ACPPNs prepared by a two-step procedure

We prepared ACPPNs by a two-step procedure with a low (<20 mmol) amount of phosphorus element (P) or one-step procedure with a high (≥20 mmol) amount of P. For an atomic ratio of Ca/P = 0.1 with 15% of P in the form of PPI (85% in the form of Pi), we have obtained amorphous calcium phosphate nanowires by adding 10 mmol P in two steps, that is, 5 mmol by 5 mmol sequentially. The two-step procedure facilitates control over the pH of the reaction solution. As shown in Fig. 1A, nanowires with average diameters of 2.8 (n = 100) although thicker ones (e.g., diameters >10 nm) are also observed, and lengths of up to hundreds of nanometers are formed. Besides, nanoparticles with irregular shapes are obtained as well. In cryogenic transmission electron microscopy (Cryo-TEM) micrographs of the sample in the reaction solution, we also observe the nanowires, proving that this morphology is formed in the solution, rather than during the subsequent sample preparation (Fig. 1B). The electron diffraction (ED) pattern (inset of Fig. 1A) of the area with nanowires (labeled by dashed circle) exhibits no distinct reflections, indicating the amorphous character of the product, which is also confirmed by high resolution TEM (Fig. S1) and X-Ray Diffraction (XRD, inset of Fig. 1A). We note that a nanowire morphology of ACaPi or ACaPPI has never been obtained before, i.e., without external regulations such as additives, templating, etc., and is not seen in other amorphous inorganic materials either, to the best of our knowledge. Energy dispersive spectroscopy (EDS) (Fig. 1C) and XPS (Fig. S2) proves the existence of Ca and P elements. Fourier transform infrared spectroscopy (FTIR) further verified the presence of PPI (P2O7^4-) in the ACPPNs due to the asymmetric vibrational bands of P-O-P at wavenumbers of 918 and 739 cm^-1 and that of PO3 at 1146 cm^-1, which is consistent P2O7^4- in ACaPPI (Fig. 1D). The presence of Pi (PO3^- or HPO3^2-) is also confirmed by the asymmetric stretching frequency of PO4 at 1097 cm^-1, agreeing with the bands of (H)PO4 in ACaPi as well (Fig. 1D). In comparison, when
adding the same amount of $P_e$ simultaneously in one step (rather than in two steps as above), more nanoparticles and fewer nanowires are formed, confirming that the two-step procedure is favorable for the formation of nanowires (Fig. S3). Also, importantly, the ACPPNs can keep the nanowire morphology after being stored at 37 °C even for 8 weeks without drying (Fig. S4).

Fig. 1. Characterization of ACPPNs prepared by a two-step procedure. (A) TEM micrograph. Insets: ED pattern (up) of the area marked with a dashed green circle and XRD pattern (down, two broad bumps at $2\theta$=~30 and ~45°) of the ACPPNs. No visible beam damage on the sample was observed after ED pattern capture. (B) Cryo-TEM micrograph with magnified local area in the inset. (C) EDS spectrum of the nanowires in the inset marked with a dashed green rectangle in the scanning transmission electron microscope (STEM) image. (D) FTIR spectra with dashed blue line labeling characteristic bands of specific groups as indicated. The assignment of the vibration modes follows the references [21].

To investigate the effect of Pi/PPi ratio on the morphology of ACPPNs, we used varied $P_e$ percentages of PPi (0, 5, 10, 15, 25, 100%) to prepare ACPPNs. For 0 % PPi,
i.e., only Pi as the Pₐ, a flake-shaped product is obtained and no nanowires are observed (Fig. S5A). From 5% (Fig. S5B), 10% (Fig. S5C), 15% (Fig. S5D), to 25% (Fig. S5E), the proportion of nanowires increases. For 100% PPI, i.e., only PPI as the Pₐ, the TEM micrograph presents a typical morphology of amorphous calcium pyrophosphate-irregular nanospheres [22]. Therefore, nanowires only form in a certain ratio range of Pi/PPI in the Pₐ; besides, neither pure Pi nor PPI leads to nanowire formation independently. The initial Ca/P atomic ratio also has an influence on the morphology of the products. When the initial Ca/P is increased from 0.1 (Fig. 1) to 1.0, i.e., 1.00 mmol Pₐ is used (other parameters are not changed), which is close to the concentration of Pₐ in human bodily fluids [23], nanowires are still formed in the above-described two-step procedure (Fig. S6A). However, only very few nanowires are visible when Ca/P=2.0 (Fig. S6B), and no nanowires are observed when Ca/P=5.0 (Fig. S6C). FTIR spectra of these three samples show that their Pi/PPI ratios are significantly increased (Fig. S6D) comparing to that prepared with Ca/P=0.1 (Fig. 1E).

We also investigated the effects of pH on the structure of the products. Based on a solution of pH=8 as discussed above, we compared the changes of morphology at different pH values. Under weakly acidic conditions (pH=6) (Fig. S7A), still the nanowires form, but they become very rare under weakly basic conditions at pH=10 (Fig. S7B). In addition to the Ca²⁺, Pi, and PPI ions, which are directly involved in the formation of ACPPNs, other counter ions (e.g., Cl⁻ and Na⁺ that are introduced to the solution from the precursor reagents CaCl₂, Na₂HPO₄, and Na₄P₂O₇) may raise concern toward their influence on the morphology of the products [12b]. When using the Ca and Pₐ sources with other counter ions, e.g., NH₄⁺, NO₃⁻ and ClO₄⁻, we still obtained the nanowire products (Fig. S8), showing that these counter ions have very little impact on the formation process.

While nanoparticles are normally accompanied by the nanowires as shown above, the introduction of additives such as polymaleic acid (PMA) and polyacrylic acid (PAA) into the reaction system significantly alters that pattern. PMA and PAA are typical polyelectrolytes with abundant carboxylate groups and normally used to mimic non-collagenous proteins when studying biominalization [24]. As shown in Fig. 2, in the presence of either PMA (Fig. 2A) or PAA (Fig. 2B), nanowires with average diameters of 2.8 (n = 100) although thicker ones (e.g., diameters >10 nm) are also observed, and lengths of up to hundreds of nanometers are prevalent in the products, while nanoparticles are hardly visible by TEM observation. The predominance of nanowires in the products may be attributed to an inhibitory effect of PMA and PAA against nucleation and subsequent solid growth, as precipitation occurs much later than in the absence of PMA and PAA (~5 min vs. 0 min). We verified the amorphous phase by ED and XRD patterns (insets of Fig. 2A and 2B, Fig. 2C), and the presence of Ca and P elements by XPS (Fig. S9). In the FTIR spectrum (Fig. 2D), apart from the presence of Pi and PPI due to the same bands as Fig. 1E, the existence of polymer is also proved by the stretching frequency of the carboxy ion (COO⁻) at 1409 cm⁻¹ (Fig. 2D).
Fig. 2. Characterization of ACPPNs prepared in the presence of nucleation inhibitors. (A, B) TEM images of ACPPNs prepared in the presence of PMA (A) or PAA (B). (C, D) XRD pattern (C) and FTIR spectrum (D) of ACPPNs prepared with PMA.

**ACPPNs prepared by a one-step procedure**

We further increased the amount of P<sub>e</sub> to ≥20 mmol (without additives) to investigate nanowire formation in the one-step procedure. As the amount of P<sub>e</sub> is much higher than that of Ca, the effect of precipitation on pH is negligible, and it is not necessary to adjust the pH during the reaction. Therefore, the one-step method can be used when adding high (≥20 mmol) P<sub>e</sub> amounts. The total P<sub>e</sub> content is increased to 20.0 and 40.0 mmol, while maintaining the proportion of PPI the same as above. In this case, the fraction of nanowires increases significantly, showing nearly pure nanowires (Fig. 3). Typically, when the total P<sub>e</sub> content is 40.0 mmol, nearly pure nanowires with a width of 3.0 ± 0.7 nm (n = 100, single nanowire or the ones at the edge of aggregates are used for measurements) and lengths of up to tens of nanometers are formed (Fig. 3B). The elemental map recorded by EDS confirms the presence of elements P and Ca (Fig. S10). In this case of high P<sub>e</sub> concentration, the stepwise addition of P<sub>e</sub> seems to have little (or even adverse) influence on the product morphology (Fig. S11). Again, ED and XRD patterns confirm the amorphous phase (insets of Fig. 3A and B, Fig. 3C). The FTIR spectra in Fig. 3D show that the intensity ratio of bands at 1146 cm<sup>-1</sup> (PO<sub>3</sub> stretching) and 1097 cm<sup>-1</sup> stretching (PO<sub>4</sub>) is significantly higher than that in Fig. 1E, indicating the increase of PPI percentage in P<sub>e</sub>, which is also confirmed by nuclear magnetic resonance (NMR) measurement as discussed in the following.
Fig. 3. Characterization of ACPPNs prepared by a one-step procedure. (A, B) When increasing the P<sub>e</sub> content to 20.0 mmol (A) and 40.0 mmol (B), nearly pure nanowires are obtained. (C) XRD patterns with two broad bumps at 2θ=~30 and ~45°. (D) FTIR spectra with dashed blue line labeling characteristic bands of specific groups as indicated.

Microstructure of ACPPNs

In addition, we analyzed the products by solid state NMR spectroscopy (Fig. 4). The single pulse 31P MAS NMR spectra show two resonances with chemical shifts of ~2.6 ppm and ~6.5 ppm, which can be assigned to Pi (PO<sub>4</sub><sup>3-</sup> or HPO<sub>4</sub><sup>2-</sup>) and PPI (P<sub>2</sub>O<sub>7</sub><sup>4-</sup>) groups, respectively (Fig. 4A) [25]. The molar ratio (P<sub>ratio</sub>) of P<sub>e</sub> in Pi and PPI for the samples is calculated by integrating the corresponding Guassian peaks after deconvolution as shown in Fig. 4A. In combination of the P<sub>ratio</sub> value and TEM results, it can be concluded that the increase of the fraction of nanowires is consistent with the higher PPI content in the products, indicating that the formed nanoparticles have more Pi while the nanowires include more PPI. In the case of uniform nanowires, the P<sub>ratio</sub> remains ~1:3 regardless of different synthesis procedures (one using PMA, and the other one using 40.0 mmol P<sub>e</sub> without additives).

Subsequently, we measured the ACPPNs prepared with 40.0 mmol P<sub>e</sub> via 2D solid state nuclear magnetic resonance (Fig. 4B-E). In the 2D 1H-31P HETCOR CPMAS NMR spectra (Fig. 4B), both Pi and PPI are strongly correlated with H. We further extracted the cross-section spectra at the 31P chemical shift of Pi and PPI as indicated in Fig. 4C. They show that the PPI peak correlates with the resonance of H from adsorbed/structural H<sub>2</sub>O while that of the Pi peak correlates with H of both H<sub>2</sub>O and HPO<sub>4</sub><sup>2-</sup>. This reveals that a certain fraction of phosphates is protonated to yield HPO<sub>4</sub><sup>2-</sup>, but pyrophosphates are not. As the Ca/P molar ratio determined by ICP-OES analysis...
is 0.98, i.e., very close to 1, we conclude that nearly all of the Pi are protonated in this sample. So, with these results and TG measurement (Fig. S12), the chemical formula of the nanowire is estimated to be CaHPO$_4$·(Ca$_2$P$_2$O$_7$)$_{1.5}$·6.5H$_2$O.

Fig. 4. Solid state nuclear magnetic resonance analysis of ACPPNs. (A) $^{31}$P MAS NMR spectra. The nanowires are prepared by a two-step procedure (top), by a one-step procedure with 40.0 mmol P$_e$ (middle), and with PMA (bottom), respectively. Black solid curve: original spectrum; blue and green curve: Gaussians from the deconvolution of the original spectrum; red dashed curve: the sum of the Gaussians. The $P_{ratio}$, ratio of P$_e$ in Pi and PPi, is shown for the corresponding spectrum of each sample. Dashed rectangles indicate the chemical shifts attributed to Pi and PPi as indicated. (B) 2D $^1$H-$^{31}$P HETCOR CPMAS NMR spectra of ACPPNs prepared with 40.0 mmol P$_e$ in one-step. (C) Extracted cross-section spectra at the $^{31}$P chemical shift of Pi and PPi as indicated in the dashed line of (B). Dashed rectangles show the resonance of H from H$_2$O and HPO$_4^{2-}$, respectively. (D, E) $^{31}$P-$^{31}$P NOESY CPMAS NMR spectra at mixing times of 6s (D) and 12 s. (F) Schematic illustration of the spatial composition of ACPPNs, where an CaPPI inner space is coated by CaPi to form a core-shell structure.

Further insight into the relationship of Pi and PPi is obtained by $^{31}$P-$^{31}$P NOESY CPMAS NMR spectra (Fig. 4D, E). At a mixing time of 6 s, no exchanges between the P$_e$ from Pi and PPi are observed (Fig. 4D). However, when extending the mixing time to 12 s, which is still very short, exchanges (positive) peaks are clearly observed (Fig. 4E). This result shows that Pi and PPi are separately distributed at independent sites, but stay very close to each other. This anisotropy can be further corroborated by STEM-EELS: data obtained on a short rod (Figure S13) indicates fundamental changes in the coordination of P$_e$ within inner and outer spaces of ACPPNs, which is consistent with the spectra of ACPi and ACPPi, and thus agree with the anisotropic distribution of Pi and PPi. Therefore, we propose the composition of ACPPNs as Fig. 4F, which displays that the CaPPI is in inner space which is coated by CaPi, forming a core-shell structure, as suggested by additional insights discussed in the following.
Fig. 5. Study of the ACPPNs formation mechanism. (A, B) Cryo-TEM images of the formed species in the solution at 5 min (A) and 10 min (B) during the PMA involved process. The magnified area indicated by dashed circles highlight single nanocluster (red), short nanorods (blue), and long nanowires (green) formed by oriented aggregation in 1-D. (C-F) TEM images of the formed species in the solution at different times during the 40.0 mmol P₆ involved process as indicated.

**Formation mechanism of ACPPNs—oriented growth**

We investigated the formation process of ACPPNs by tracking the evolution of the formed species in the reaction solutions. As the process takes about 5 minutes in the presence of PMA to form visible precipitation after adding the P₆ source, which is a much slower reaction than that without polymers (immediate precipitation), it becomes possible to characterize intermediate species of the reaction. Therefore, we first studied the formation process involving PMA by cryo-TEM. It shows that, 5 min after P₆ addition, nanoclusters with a size of ~1-2 nm are obtained, and some of them begin to aggregate or assemble into short nanorods (Fig. 5A); 10 min after P₆ addition (Fig. 5B), nanowires with a diameter of ~2 nm, which is similar to that of the former nanoclusters, are predominant in the micrographs. Moreover, in some nanowires, which should be the early formed ones, distinct boundaries in between the nanoclusters are observed. FTIR spectra show that, from the early to final states of reaction, there are no distinct changes, indicating that the chemical compositions do not significantly vary during the evolution (Fig. S14A). Therefore, for the formation process, nanoclusters with a size of...
1-2 nm are formed in the solution first, after adding $P_e$; then, they aggregate into short rods; finally, the rods grow into nanowires by attaching more nanoclusters (Fig. 6).

Fig. 6. Schematic illustration of a possible ACPPNs formation mechanism with nucleation inhibitor (PMA or PAA) and without nucleation inhibitor. In the presence of nucleation inhibitor, ions (A) react to form nanoclusters with a size of 1-2 nm (B1) firstly after adding the $P_e$ source; then, they aggregate into short rods (B2); and finally grow into nanowires (D). However, for ACPPNs preparation without nucleation inhibitor, nanoclusters are formed first (C1), then quickly aggregate into nanoparticles (C2), but finally transform into nanowires (C3) by rearrangement or a dissolution-reprecipitation process.

The formation of ACPPNs without polymer progresses so rapidly that precipitation occurs as soon as $P_e$ is added. Thus, the question whether nanowires are formed immediately or after a certain lag time spurs us to investigate the formation progress with 40 mmol $P_e$. Fig. 5C shows that only nanoparticles, which seem to be built up by nanocluster aggregation, are observed in the sample at 0 s. Over time, nanowires appear at 15 s, with their fraction increasing afterwards, and dominating at 60 s (Fig. 5D-F). This indicates that the nanowires are formed at expense of nanoparticles, the fraction of which decreases. Again, FTIR spectra show that there are no distinct changes over time indicating that the chemical compositions do not significantly vary (Fig. S14B). Therefore, in this system, nanoclusters are formed first, but instead of subsequent aggregation in 1-D in the presence of PMA, they then quickly aggregate into nanoparticles, probably due to diffusion limited aggregation. However, the nanoparticles finally transform into nanowires by rearrangement, or a dissolution-reprecipitation process (Fig. 5). It is reasonable to understand the aggregation of early formed nanoclusters: without nucleation inhibitor and stabilizer like PMA, a large number of nanoclusters is formed in a very short time, which tend to first randomly aggregate due to the high specific surface area and high surface free energy. The transformation of nanoparticles into nanowires at nearly 60 s indicates that nanowire formation should be controlled by thermodynamics rather than kinetics, that is, the
nanowires seem to be more thermodynamically favored than nanoparticles in long term, although we cannot categorically rule out that also kinetic aspects play a role.

This begs the question whether the pre-synthesized ACaPi and ACaPPi can transform into ACPPNs? In order to explore this further, we added Pi and P Pi sequentially in two steps with a time interval of 3 s (P e is 10.0 mmol, in which Pi and P Pi are 8.50 and 0.75 mmol), by which ACaPi or ACaPPi was initially formed by the first added Pi or P Pi, then further reacted with the next added P Pi or Pi. First of all, we made sure that after the same dosage of P Pi or Pi, no nanowires were produced, regardless whether it was added directly (Fig. S15A) or in two steps (Fig. S15B). However, like above, nanowires are generated in the presence of both Pi and P Pi although they are added in separate steps (Fig. S15C-D). Moreover, there are many more nanowires (Fig. S15C) when the P Pi is added in the first step, and Pi in the second (denoted as P Pi-and-Pi), than in the case of the reverse sequence of P e addition (Pi-and-P Pi) (Fig. S15D). It should be noted that when P Pi or Pi is first added, pure ACaPPi or ACaPi forms primarily, and the next addition of Pi or P Pi can only partially substitute P Pi or Pi in the products. Furthermore, we extend the time interval between P Pi and Pi addition and remove the excess P e source during the interval by washing with a large amount of H2O to avoid effects of the first-added excessive P e. Again, nanowires form with P Pi in the first step (P Pi-and-Pi, Fig. S15E), but they are nearly invisible with P e added in reverse sequence (only crystalline CaPi, Fig. S15F). All of the above reveals that ACaPPi or ACaPi can transform into ACPPNs although their performance varies under different conditions. When tracking the transformation process with P e addition in the sequence of P Pi-and-Pi by Cryo-TEM, we find that, nanowires are formed via the transformation of nanoparticles after adding Pi (Fig. S16).

In essence, as discussed further above, the nanowire mainly consists of ACaPPi rather than ACaPi, but only the addition sequence of P Pi-and-Pi yields nearly pure nanowires (Fig. S15C). This further confirms that, (1) in addition to P Pi, Pi is indeed included in nanowires as well; and (2) excludes the possibility that P Pi acts as the surfactant to induce the nanowire formation because P Pi has been consumed in the first step of addition in the P Pi-and-Pi sequence. Altogether, these observations also strongly suggest that CaPPi forms the inner core of the ACPPNs, supporting the structural model of ACPPNs in Fig. 4F.

It has been established that, in crystals, the energy differences between different facets, drives the preferential growth according to Wulff’s rule [16], leading to certain facets exposed on the surface of crystals, forming distinct planes, angles, and edges. However, it is surprising that ACPPNs, as an amorphous phase, grow into this 1-dimensional structure instead of nanospheres or irregular aggregates of nanoparticles without any external regulation, such as templates, surfactants, electricity, etc. We also exclude the effect of mechanical shear stress of stirring, by obtaining nanowires without stirring after P e addition (Fig. S17). Generally, the amorphous solids, exhibit order in their microstructure within a very short range [19], where the atoms and ions are, on average of nanometer scale and longer, uniformly distributed in the solids [15c, 18]. So,
when the amorphous solids grow by either attaching nanosized particles or atoms/ions, there are no energy differences between each spatial direction, that is, all directions are equally favored by the atoms and ions during attachment. This should, like in the case of other amorphous solids, lead to the morphologies of nanospheres or irregular aggregates of nanoparticles. Thus, the fact that we obtain nanowires with such high aspect ratio in our study expands this conventional view.

Considering that various preparation conditions (one-step, two-step, with or without polymers, \(P_e\) addition of PPi-and-Pi), and different intermediate states (nanoclusters and nanoparticles with different compositions) all result in ACPPNs formation, the growth of the nanowire should be mainly driven by its inherent properties. Therefore, it is reasonable to propose that, like in crystals, the anisotropy of the ACPPNs microstructure causes the oriented growth. As discussed above, the anisotropic distribution of Pi and PPi within the nanowires has been corroborated by \(^{31}\text{P}\) MAS NMR spectra (Fig. 4) and STEM-EELS (Fig. S13), and is consistent with the fact that the specific addition sequence of PPi-and-Pi is important for yielding nearly exclusively nanowires (Fig. S15). In this case, as shown in the Figure S18, during the growth of nanowire with such core-shell structure (Fig. 4F), the surfaces chemical environments in parallel and vertical directions are different (dominated by the distinct components ACaPPi vs ACaPi-ACaPPI, respectively). Thus, the building units (ions or nanoclusters) are allowed to attach on the nanowire in a preferential rather than random way, e.g., along the nanowire, based on the energy differences.

As for the possible anisotropy in the nanoclusters, we can hardly determine it by experimental characterizations due to their transient state in the solution and very small size. Actually, it is believed that some amorphous solids are composed of clusters with specific structures whose sizes are comparable to the short order range, for example, the so-called Posner’s cluster or CHPC cluster \([11]\). Indeed, the hydrogen phosphate-based CHPC bears the potential to exhibit anisotropy due to its more flexible structure, as opposed to the Posner’s cluster. Also, anisotropy of a special ACaPi building block stabilized by a small molecule was indicated by Tang et al. \([13c, 13d]\). In their work, linear ion oligomers of amorphous calcium phosphate were prepared, where the chemical environment of ends and middle segments are obviously different. However, oligomer attachment did not occur in 1-dimension during the growth, and nanowires were not observed. As for ACaPPI, its microstructure has been much less studied than that of ACaPi. Although an order range of 8 Å was indicated by the pair distribution function pattern \([5c]\), potential anisotropy was not studied further. In the nanoclusters-involved nanowire growth, beside the anisotropic ACPPPNs, Ca, Pi, PPi, and H\(_2\)O should also have formed anisotropic building blocks, i.e., the nanoclusters, with a heterogeneous distribution of components. This anisotropy may give rise to an amphiphilic like property of the nanoclusters, which subsequently self-assemble. That is, the nanoclusters can be regarded as inorganic analogues of amphiphilic organic diblock copolymers, or surfactants. In this sense, our observations may resemble the formation of core-shell structured cylindrical micelles: as in the case of amphiphilic diblock
polymers/surfactants with two kinds of segments (corresponding to ACaPPi and ACaPi in the nanoclusters), these units self-assemble into cylindrical micelles (corresponding to ACPPNs), in which one of the segments makes the core of micelles (ACaPPi) and the other one forms the shell (ACaPi) \[26\]. Please note that the formation of ACPPNs is not dependent on the presence of organic amphiphiles, but an analogous, purely amorphous inorganic phenomenon.

Moreover, the rather slow assembly velocity of building blocks seems necessary for the nanowire formation. Otherwise, the building blocks do not have sufficient time for direction selection to form nanowires. In the case of PMA and PAA involved reaction, it takes more than 5 minutes to initiate nucleation and further 10 minutes to finish the assembly; in the case of 40 mmol P_{e} involved reaction, 1 min is needed to transform nanoparticles into nanowires. This also inspires us that, a slow transformation from one amorphous solid to another amorphous one, may proceed via oriented growth as well in other materials.

**Conclusion**

We have prepared amorphous calcium phosphate-pyrophosphate nanowires (ACPPNs) in aqueous solution via a two-step procedure with low (<20 mmol) amount of P_{e} or one-step procedure with high (≥20 mmol) amount of P_{e}. It is composed of amorphous calcium phosphate and amorphous calcium pyrophosphate and displays the morphology of nanowires with average diameter of ~2-3 nm, and lengths of up to hundreds of nanometers. Both Pi and PPi are indispensable for the formation of nanowire, in which the ACaPi and ACaPPi are distributed at separated sites but stay close to each other. We propose a core-shell structure model for this nanowire, where the inner component is calcium pyrophosphate and the outer one is calcium phosphate. Further studies show that the ACPPNs form via either the preferential attachment of ~2 nm nanoclusters in 1-dimension fashion (in the presence of nucleation inhibitor), or the transformation of bigger nanoparticles (without nucleation inhibitor). We propose that an anisotropy of the ACPPNs microstructure should drive their preferential growth, which is rarely seen in ACPs and other amorphous solids. This proves that, unlike the conventional view, amorphous solids can form via oriented growth, expanding the conventional view on the structure and growth behavior of amorphous minerals. This finding may also provide a new perspective for biomineral growth in vivo, which will be studied in the future.

Due to technical limitations, characterizing the microstructure of amorphous solids remains a great challenge. Therefore, the specific arrangements of the ions in ACPPNs were not determined in this study. Although we proved the anisotropy of ACPPNs, direct evidence for our proposal on the structure of ACPPNs still needs further exploration in the future. We also would like to remind that, during this study, we found significant content of impurity PPi in the chemical Na_{2}HPO_{4} (analytical grade) from different commercial suppliers, which may have impacted or will further affect the results of researches.
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