

Crystallization

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Distinct Short-Range Order Is Inherent to Small Amorphous Calcium Carbonate Clusters (< 2 nm)

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Abstract: Amorphous intermediate phases are vital precursors in the crystallization of many biogenic minerals. While inherent short-range orders have been found in amorphous calcium carbonates (ACCs) relating to different crystalline forms, it has never been clarified experimentally whether such orders already exist in very small clusters less than 2 nm in size. Here, we studied the stability and structure of 10,12-pentacosadiynoic acid (PCDA) protected ACC clusters with a core size of ca. 1.4 nm consisting of only seven CaCO_3 units. Ligand concentration and structure are shown to be key factors in stabilizing the ACC clusters. More importantly, even in such small CaCO_3 entities, a proto-calcite short-range order can be identified but with a relatively high degree of disorder that arises from the very small size of the CaCO_3 core. Our findings support the notion of a structural link between prenucleation clusters, amorphous intermediates, and final crystalline polymorphs, which appears central to the understanding of polymorph selection.

Amorphous intermediate phases, commonly found in the early stages of mineral formation, are important precursors in the crystallization of many biogenic minerals such as sea urchin spicules^[1] and plant cystoliths.^[2] In recent years, plenty of evidence has been found for a “nonclassical” nucleation route of minerals involving prenucleation clusters as intermediate precursors.^[3–5] Stable prenucleation clusters were first discovered in CaCO_3 precipitation,^[3] and were also reported for other systems like calcium phosphate,^[6] magnetite,^[7] and silica.^[8] In the case of CaCO_3 , according to the hypothetical prenucleation cluster pathway, crystallization proceeds through the formation of amorphous calcium carbonate (ACC) by the aggregation of nanodroplets that

directly emerge from prenucleation clusters upon phase separation, and subsequent transformation into crystals.

The CaCO_3 prenucleation clusters with a size between 0.6–1.1 nm^[9] and 2 nm^[3] were shown by computer simulation to be disordered, flexible, and strongly hydrated liquid-like ionic polymers with a dynamic topology of chains, branches, and rings.^[10] Titration results of CaCO_3 precipitation implied a link between binding strength in prenucleation clusters and the type of postnucleation amorphous phase, suggesting that short-range order discovered in ACCs^[11–18] may already exist in the prenucleation clusters.^[3] However, experimental investigation or verification of the structure of CaCO_3 clusters is quite challenging. First, it requires stopping the process of precipitation at the initial stages and isolating prenucleation clusters. Second, if the cluster can be stabilized, the hybrid structure must be well-defined for easy analysis. A few efforts have been made to stabilize and elucidate the CaCO_3 cluster structure. For example, the addition of silica was shown to be able to effectively suppress the nucleation of CaCO_3 clusters even at high supersaturation.^[19] However, the probable existence of silica particles and silica-associated clusters as well as other salts made the isolation and structural characterization of CaCO_3 clusters difficult. Poly(acrylic acid) appeared to also stabilize ACC nanograins sized 2–3 nm, but the formed nanocomposite structure renders isolation and detailed analysis of CaCO_3 clusters improbable.^[20]

In our previous study, we found a unique amphipathic ligand, 10,12-pentacosadiynoic acid (PCDA), that can effectively stabilize ACC with a very small core size of ca. 1.4 nm, which was used for subsequent investigation of its crystallization behavior.^[21] Preliminary characterizations showed that the particle (named ACC cluster) might have a micellar structure involving an ACC core decorated by PCDA chains with the structural composition of $(\text{CaCO}_3)_7(\text{H}_2\text{O})_4(\text{PCDA})_3$. It is noted that the size of the CaCO_3 core in the ACC cluster is quite comparable to that of prenucleation clusters in aqueous solution, and the number of CaCO_3 units in the cluster is close to that in the primitive rhombohedral unit cell of calcite (consisting of six ion pairs).^[15,22] Therefore, it would be interesting to know whether a distinct short-range order still exists in such a small CaCO_3 entity.

Given the highly dynamic character of CaCO_3 prenucleation clusters in aqueous solution,^[10] we examined the stability of the PCDA-protected ACC cluster towards crystallization. Unexpectedly, at room temperature, the ACC clusters can be stored in toluene or in dry state for at least six months without any change. Furthermore, the ACC clusters are very stable under the action of heat (100 °C for 4 days, Figure S1), water vapor, and several additives, or under electron-beam irradiation in the transmission electron microscope (TEM).

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To verify the specific role of PCDA in stabilizing the ACC cluster, we studied the effect of PCDA concentration on producing ACC clusters. As shown in Figure 1, only when the

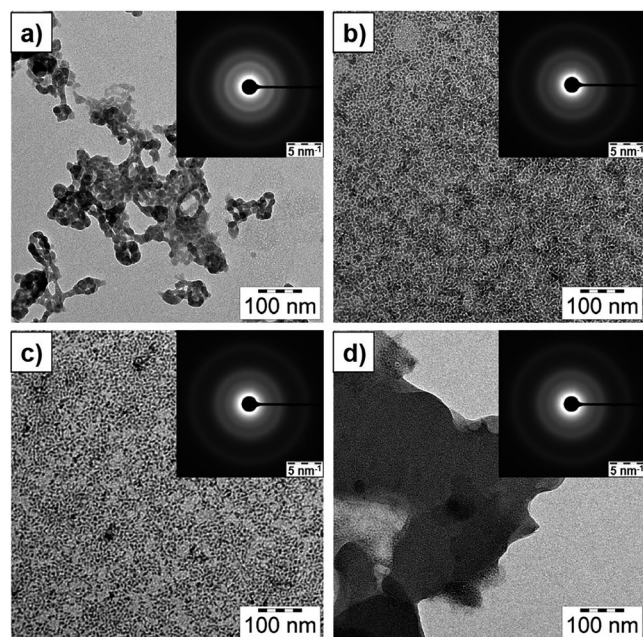


Figure 1. TEM images and corresponding SAED patterns of CaCO_3 synthesized at different concentrations of PCDA: a) 2 mM; b) 3 mM; c) 4 mM; d) 10 mM.

concentration of PCDA is 4 mM can perfect clusters be formed. A lower concentration of PCDA (2 mM) is not able to stabilize small clusters, resulting in large amorphous particles (10–15 nm), while higher levels of PCDA (10 mM) often result in a hybrid layered structure of PCDA and clusters. A transient phase can be observed when the concentration of PCDA is 3 mM. Apparently, the PCDA concentration is a key factor for the formation of stable ACC clusters. This is consistent with the fact that the optimized concentration of PCDA (4 mM) corresponds to the feed ratio of $[\text{Ca}^{2+}]/[\text{PCDA}]$ of 2.3–2.5 which is in good accordance with the proposed molecular formula of the ACC cluster, $(\text{CaCO}_3)_7(\text{H}_2\text{O})_4(\text{PCDA})_3$, for nearly 100% conversion in terms of Ca^{2+} . While it is still not clear why PCDA can stabilize such small ACC clusters, the number of CaCO_3 units—seven—finds support in recent ab initio simulations where the stabilization energy per monomer appears to reach a plateau at this size.^[23]

Moreover, control experiments with additional ligands (Figures S2,3) show that besides PCDA, oleic acid and sodium bis(2-ethylhexyl)sulfosuccinate (NaAOT) can stabilize a small amount of clusters. It is speculated that only fatty acids with long alkyl chains and low chain order effectively stabilize the CaCO_3 clusters, especially when we compare the chemical structures of oleic acid and stearic acid which have the same chain length but different degrees of structural saturation. Long alkyl chains are known to be favorable for the formation of stable self-assembled monolayers with high ligand coverage and can provide substantial barriers to water

and ion penetration.^[24] The diacetylene group or double bond existing in the long alkyl chain may interrupt the chain order during the binding process on CaCO_3 , which consequently hinders the formation of ordered CaCO_3 crystallites.

As the diacetylene group of PCDA is easily polymerized by light irradiation,^[25] it is essential to know whether the PCDA monolayer polymerizes during reaction and posttreatment. The similarity between the UV/Vis absorption of ACC clusters and PCDA in hexane (Figure 2a) shows that the

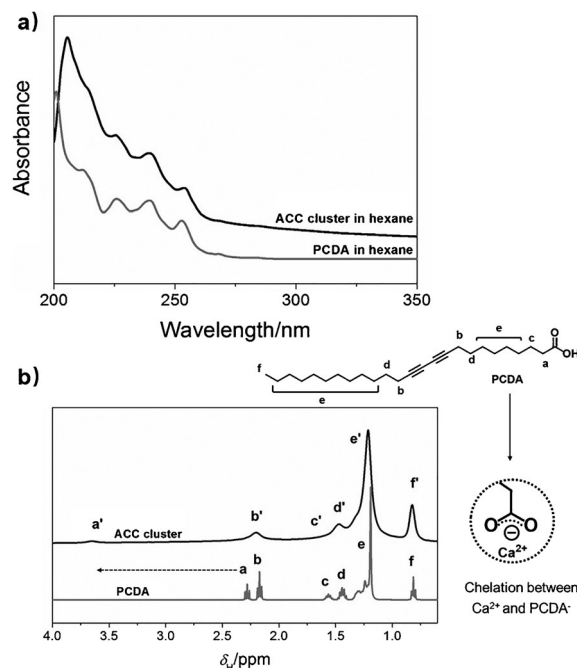


Figure 2. a) UV/Vis spectra of ACC clusters and PCDA in hexane. b) ^1H NMR spectra and corresponding resonance assignments of ACC clusters and PCDA in $[\text{D}]\text{chloroform}$. a, b, c, ... represent hydrogens in PCDA while a', b', c', ... represent hydrogens in the ACC cluster.

PCDA chains in the ACC clusters are still in their monomeric form after binding on the CaCO_3 core. No absorption in the visible region can be observed, corresponding to the good transparency of dispersions of ACC clusters due to their small size. Raman spectroscopy further confirmed the monomeric form of PCDA chains (Figure S4). Compared to PCDA, the ^1H NMR spectrum of ACC clusters in $[\text{D}]\text{chloroform}$ exhibits a resonance peak broadening effect, and a large shift of H_a (to $\text{H}_{a'}$) corresponding to the methylene group adjacent to COOH , indicating the chelation between Ca^{2+} and PCDA.

That the PCDA chains are bound to Ca ions within clusters is also evidenced by the shift of the $\text{C}=\text{O}$ stretching band from COOH (1690 cm^{-1}) to COO^- (1547 cm^{-1}) in the IR spectra (Figure 3). The broad band between 3600 and 3200 cm^{-1} is due to the structural water in the cluster, suggesting that the CaCO_3 core is hydrated. Interestingly, by comparison with synthetic ACC with known composition, the ACC cluster core corresponds to $\text{CaCO}_3 \cdot 0.57\text{H}_2\text{O}$ (Figure S5), which is also in accord with the proposed molecular formula of the ACC cluster, $(\text{CaCO}_3)_7(\text{H}_2\text{O})_4(\text{PCDA})_3$.^[21] The alkyl chains of PCDA are partly converted to gauche

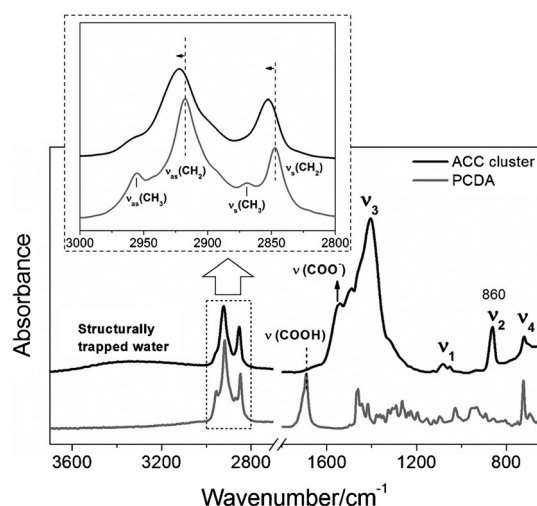


Figure 3. ATR-FTIR spectra of ACC clusters and PCDA. The inset shows an enlarged view of the C–H stretching region shown in the dashed box.

conformations upon binding to the CaCO_3 core, as evident from the shift of the C–H stretching bands of the ACC cluster to larger wavenumbers (inset in Figure 3).^[26] More importantly, the ν_1 – ν_4 bands can be used to relate the structure of the ACC cluster to distinct amorphous forms.^[16,17] The ν_1 and ν_4 bands are partially superimposed by vibrational modes of PCDA, but the ν_2 and ν_3 modes of ACC cluster at 860 cm^{-1} and $1399/1462\text{ cm}^{-1}$ (double band) are very close to that of proto-calcite ACC at 862 cm^{-1} and $1392/1462\text{ cm}^{-1}$,^[16] respectively. This indicates the small ACC clusters exhibit a proto-calcite short-range order in the CaCO_3 core.

To further prove the existence of proto-calcite order in the ACC clusters, we performed solid-state ^{13}C NMR analysis. Note that the sample for NMR was ^{13}C -enriched, so as to avoid any interference from PCDA chains. The clusters were prepared by changing the CO_2 supply from $(\text{NH}_4)_2\text{CO}_3$ decomposition to the rapid release of $^{13}\text{CO}_2$ via addition of HCl to $\text{Na}_2^{13}\text{CO}_3$. TEM, IR, and ^1H NMR studies all indicate the similarity of the ACC clusters, independent of the supply source of CO_2 (Figures S6,7). As shown in Figure 4a, a broad resonance centered at 168.5 ppm can be observed in the solid-state ^{13}C NMR spectrum of the ACC clusters, which agrees with that of both calcite and proto-calcite ACC (168.7 ppm) within experimental accuracy.^[16] The full width at half maximum (FWHM) of ca. 3.8 ppm for the ACC clusters is even larger than that of proto-calcite ACC (3.6 ppm).^[16] This indicates that the CaCO_3 core of the ACC clusters indeed exhibits a proto-calcite structure but with a relatively high degree of disorder that likely arises from the very small size of the ACC cluster core. Despite the discovery of several proto-crystalline forms of short-range order in ACC (or polyamorphism),^[16–18,27,28] the here reported finding of the existence of short-range order in very small ACC clusters less than 2 nm has never been reported, which also supports the relevance of the preformed short-range order in prenucleation clusters.^[3]

We further employed Ca K-edge X-ray absorption analyses to investigate the internal structure of the ACC

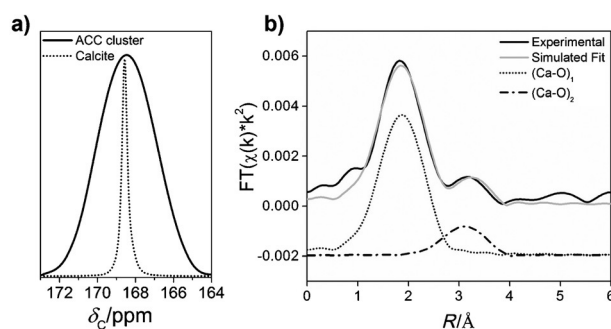


Figure 4. a) Solid-state ^{13}C NMR spectra of ACC clusters and calcite. The resonance at 168.5 ppm suggests that the CaCO_3 core in the ACC clusters exhibits a proto-calcite structure. The FWHM is ca. 3.8 ppm . b) Fourier transform of Ca K-edge EXAFS plotted in the R -space. The fitted curves of the first two Ca–O coordination shells are shown at the bottom with an offset of -0.002 units.

clusters. In the Ca K-edge extended X-ray absorption fine structure (EXAFS) spectrum of the ACC clusters (Figure 4b), a large Debye-Waller factor (σ^2) of $0.015 \pm 0.004\text{ Å}^2$ for the first shell and a small coordination number of 2 for the second shell (EXAFS fitting results in Table S1) further demonstrate a small CaCO_3 cluster size and a rather disordered nature of Ca sites with a mix of Ca–O coordination geometries and bond lengths.^[16,29,30] Comparison of Ca K-edge X-ray absorption near-edge structure (XANES) spectra of ACC cluster, proto-calcite ACC and calcite further support that ACC clusters are relatively disordered (Figure S8). Furthermore, a simulated Ca K-edge XANES spectrum for the optimized cluster model^[21] shows the emergence of a prominent near-edge feature at $2\text{--}3\text{ eV}$ compared with calcite again indicating the small size of the ACC clusters with a high degree of disorder.

It should be noted that, despite the confirmed existence of short-range order in such small ACC clusters, it remains unclear how the short-range order arises from. The ACC cluster might have a hydrated chain-like structure as shown in the simulation of prenucleation clusters.^[10] Recently, Rez et al. proposed a nanocrystallite model for ACC where randomly oriented nanocrystals with a size of ca. 1 nm could give rise to coherent diffraction profiles that are characterized as amorphous, and water molecules fill in the spaces between the distorted nanocrystallites.^[22] However, in the case of the present ACC clusters, the lack of CaCO_3 units for constructing a single nanocrystalline calcite unit cell and the fact that water molecules can be present only in the cluster core, due to the hydrophobicity of the ligand, exclude this possibility. Owing to the rather small size of the CaCO_3 core, a radial water distribution, with the outer shell being more hydrated than the inner core as observed for larger ACC particles,^[31] is not expected for these ACC clusters.

Altogether, in this study, we elucidated the principles for producing stable ACC clusters through ligands (proper concentration, long alkyl chain and low chain order) and in detail characterized the ACC cluster protected by PCDA chains. What is more important, for the first time we discovered the inherent ACC-like short-range order of proto-calcite existing in ACC clusters with a size less than

2 nm and only seven CaCO_3 units, but with a high degree of disorder due to the very small size, which is reminiscent of the structure of prenucleation clusters in the solution case. Our findings demonstrate that species which contain as few CaCO_3 units as are approximately contained in the primitive unit cell of calcite can already have a proto-calcite short-range order. This has far reaching implications for our understanding of crystallization and polymorph control, since structural information can be encoded already in smallest clusters with sizes as small as prenucleation clusters.[SS1]

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