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The effect of aspartic acid and glycine on amorphous calcium carbonate (ACC) structure, stability and crystallization

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Abstract

The effect of organic molecules on CaCO₃ crystallization, in particular on the formation of the initial amorphous calcium carbonate (ACC) phase, is poorly understood despite this knowledge being crucial for designing biomimetic compounds with specific function, strength and stability. We monitored ACC crystallization in the presence of varying concentrations of aspartic acid (ASP) and glycine (GLY). We observed an increase in ACC lifetime with increasing amino acid concentrations and showed that the amino acid molecules sorbed onto the ACC particles. However, little if any difference in composition and atomic structure or the so formed ACC was observed. Similarly, the crystallization pathway of ACC via vaterite and calcite although delayed, was only slightly affected by the added amino acids. The only exemption was at the highest tested ASP concentration where ACC formation was inhibited. The calcite crystals that formed in the presence of ASP had rounded edges and rough surfaces, features that are not observed for the pure, inorganic calcite or calcite formed in the presence of GLY. Overall, the results suggest that the amino acids affected ACC lifetime through the inhibition of crystal nucleation and growth, more so in the presence of ASP than GLY.

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1. Introduction

The formation of calcium carbonate (CaCO_3) minerals in natural environments frequently occurs in the presence of organic molecules. These are known to strongly modify CaCO_3 crystal structure, morphology and properties, often leading to more resilient and stronger crystals [1,2]. Detailed understanding of how organic molecules modify CaCO_3 growth kinetics and the mechanisms that control precipitation, is therefore of great interest.

At high supersaturation, the initial steps of CaCO_3 crystallization usually occur through the formation of poorly ordered amorphous CaCO_3 (ACC). This phase is often short lived and quickly transforms to more stable CaCO_3 phases such as vaterite, calcite and aragonite [3]. A great deal is known about how the stability of ACC and its crystallization kinetics are affected by temperature, pH and inorganic additives [4-6]. However, less is known about how organic molecules affect the atomic structure, composition, crystallization pathways and stability of ACC. Here, we investigated the role of two amino acids, aspartic acid (ASP) and glycine (GLY) in ACC crystallization using time resolved UV-Vis spectrophotometry combined with synchrotron radiation pair distribution function (PDF) analyses, X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), thermogravimetric analyses and electron microscopy.

2. Methods

CaCO₃ synthesis and crystallization: A 13 mM CaCl_2 solution and several 13 mM Na_2CO_3 solutions with added amino acids (ranging from 2.6 to 87 mM ASP or GLY) were prepared using reagent grade chemicals and ultrapure deionized water (MilliQ). All amino acid containing carbonate solutions were pH adjusted to 11.2 (2 M NaOH) to match the pH of the pure Na_2CO_3 solution. To follow ACC formation and crystallization processes using UV-Vis spectrophotometry, equal volumes of a calcium and a carbonate (\pm amino acid) solution were mixed inside a plastic cuvette placed in the spectrophotometer. During measurement, the solutions were continuously stirred and the absorbance (at 450 nm) monitored at 1 second intervals. To obtain compositional and structural information about ACC through XPS and PDF analyses ACC was synthesized by mixing 20 ml of the calcium bearing solution into the carbonate solution (\pm ASP or GLY) followed by rapid separation of solid and solution using vacuum filtration. Immediately after filtration, the solid was rinsed with isopropanol to remove water and then quickly dried by blowing air onto the solid, following the same method described by Rodriguez-Blanco et al. [7].

CaCO₃ characterization: The local atomic structure was determined using synchrotron radiation PDF analysis at beamline 11-ID-B (58.6 keV, $\lambda = 0.2114 \text{ \AA}$) at the Advanced Photon Source, Argonne, USA. Kapton capillaries were filled with dried ACC samples and measured for 5 minutes. Station set up, calibration procedures and standard data treatment with Fit2D and PDFGETX2 are detailed elsewhere [8,9]. The surface composition of ACC was quantified with XPS using a Kratos Axis UltraDLD system (AlK α X-ray source, $h\nu = 1486.6 \text{ eV}$, power = 150 W) and fast freezing techniques [10]. CaCO_3 polymorphs were identified by XRD (Bruker D8, Co K $\alpha_{1,2}$ radiation, $0.02^\circ \text{ step}^{-1}$ from 10 to $70^\circ 2\theta$, 1° min^{-1}) and imaged using scanning electron microscopy (SEM, FEI Quanta 3D, 10 kV). To obtain solid samples, at various times during the crystallization reaction the reacting aqueous suspensions were filter-quenched, the solids rinsed with isopropanol and dried prior to analyses.

3. Results and Discussion

The effect of amino acid on ACC crystallization in solution was investigated by monitoring the time dependent change in the solution absorbance using UV-Vis spectrophotometry (Fig. 1A). Previous work has shown that with this method, the formation of the initial amorphous CaCO_3 precursor, as well as its subsequent transformation to crystalline phases and the aggregation and settling of the growing crystals can be followed [11]. Figure 1B&C show the results of the spectrophotometric analyses for aspartic acid (ASP) and glycine (GLY) at concentrations between 2.6 and 87 mM. Under all tested conditions (except 87 mM ASP), instantaneous ACC precipitation was observed as illustrated by an immediate increase in absorbance when the calcium and the carbonate solutions were mixed, at $t = 10 \text{ s}$. Little to no difference was observed between the pure system and systems with added amino acids at

concentrations of 2.6 and 6.5 mM, where the ACC was stable for about 50 s. ACC lifetime increased considerably, however, at ASP concentrations of 13 mM (100 s) and 26 mM (175 s, Fig. 1B). Similarly for GLY, the ACC lifetime was prolonged but only at a concentration of 26 mM (75 s) and 87 mM (150 s, Fig. 1C). At 87 mM ASP concentrations, ACC formation was inhibited and direct nucleation and growth of vaterite and calcite were observed after an induction time of 360 s. XRD and SEM analyses also verified these crystallization trends and the effect of the amino acids on the morphology of the precipitates is shown in Fig. 2.

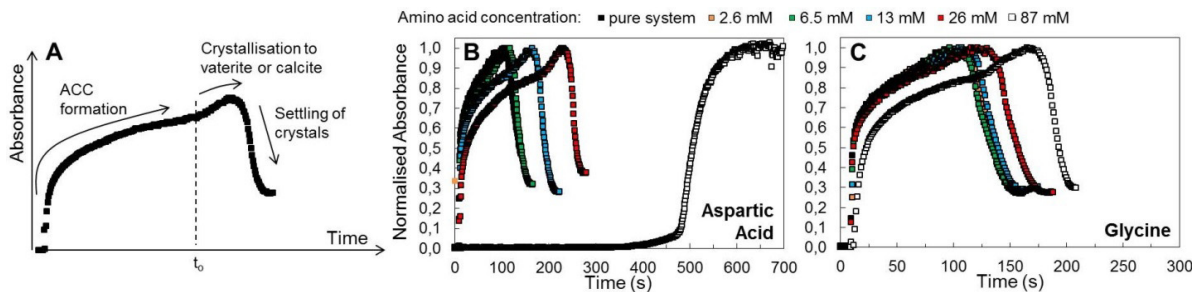


Figure 1: ACC formation and crystallization monitored by UV-Vis spectrophotometry. A) The absorbance profile for the pure system, where ACC forms first and then crystallizes to vaterite or calcite. t_0 marks the induction time before formation of crystalline CaCO_3 . B & C) Normalized absorbance (450 nm) as a function of time after mixing two solutions of 13 mM CaCl_2 and 13 mM Na_2CO_3 , in a series of solutions of ASP (B) and GLY (C).

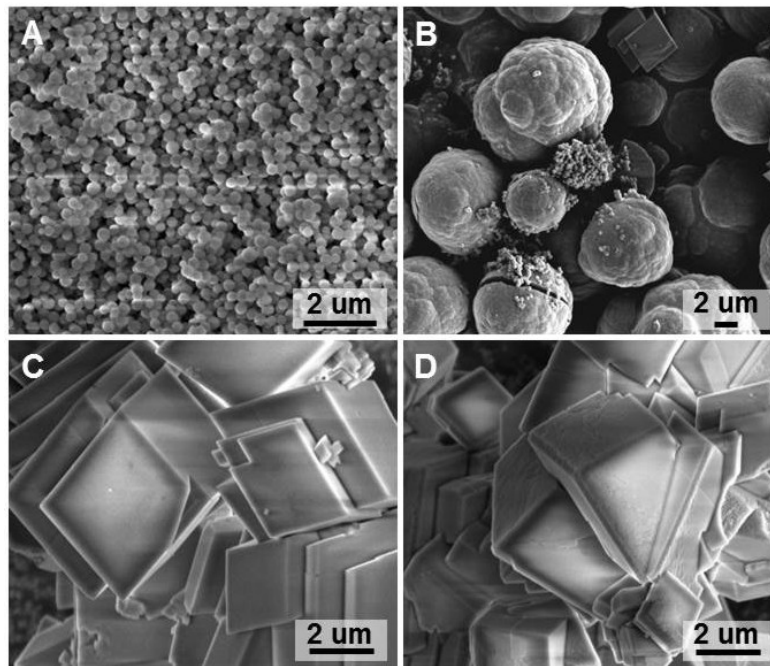


Figure 2: SEM images of precipitates from the various ACC crystallization experiments. A) Initial ACC phase in the pure system, showing ~ 300 nm diameter particles; B) Precipitate collected at peak absorbance in the 13 mM GLY system (100 seconds, see Fig. 1C), where most of the ACC has transformed to vaterite and some to calcite; C) calcite from the end of a GLY amended experiment (13 mM GLY) showing identical morphologies to the pure system with sharp crystal edges; and (D) calcite crystals from the end of an ASP amended experiment (13 mM) showing rounded edges.

In the pure system, ACC crystallized rapidly (< 2 min at $25\text{ }^{\circ}\text{C}$) to vaterite or calcite (Fig. 2A, B). It then took between 1 and 4 hours before all vaterite transformed to calcite through dissolution / reprecipitation [3,12]. In solutions with the two amino acids, ACC crystallization proceeded as in the pure system and after 4 hours, all crystals in solution were calcite (Fig. 2 C, D). Interestingly, the calcite crystals in ASP solutions did not have as sharp edges as observed for calcite formed in GLY solutions or the pure system (Fig. 2C, D), indicating that the presence of ASP affected calcite crystal growth.

SEM analyses of ACC precipitates from all experiments showed that ACC particles consisted of smooth spheres with diameter ~ 300 nm (Fig. 2A). The presence, type or concentration of the amino acid did not significantly affect the resulting ACC size or morphology. Similarly, pair distribution function analyses of these ACC precipitates showed no difference in the local atomic structure of the various ASP or GLY ACC. The PDFs for all tested ACC were identical (Fig. 3A) and displayed a structural coherence of less than $15\text{ }\text{\AA}$. Furthermore, no detectable change in water content or incorporation of amino acids was observed with thermogravimetric measurements. Consequently, structural and compositional variation is unlikely to be the reason for the longer ACC lifetime observed in the experiments with higher amino acid concentrations (Fig. 1B-D).

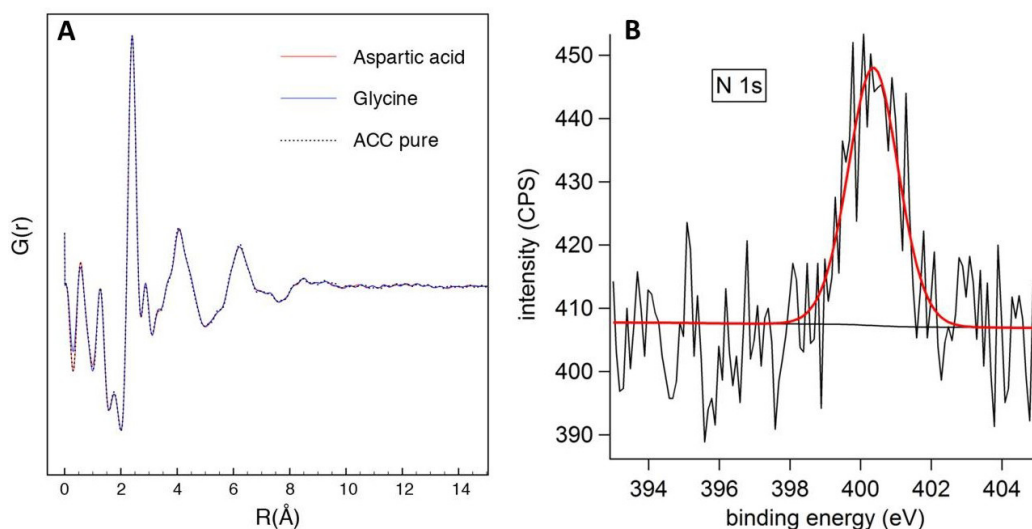


Figure 3: A) PDF of ACC synthesized by mixing 13 mM CaCl_2 with 13 mM Na_2CO_3 solutions, two of which were amended with 13 mM ASP or GLY. The PDF data of the ACC that formed in each of these solutions were identical. B) XPS spectrum showing the nitrogen peak for ASP sorbed onto the surfaces of ACC particles.

It is important to note that both ASP and GLY sorbed to the surface of ACC particles, as demonstrated by a nitrogen peak in the XPS spectra (Fig. 3B). Somewhat higher adsorption was measured for ASP (atomic % ratio $\text{N}/\text{Ca} = 0.8$) compared with GLY ($\text{N}/\text{Ca} = 0.3$) on ACC formed from solutions with 13 mM amino acid. It is however questionable if this can explain the prolonged ACC lifetime, in particular because previous studies have shown that ACC dissolution is not strongly affected by sorbed organic molecules [13]. Indeed, Ihli et al. [13] did not detect any significant change in ACC composition or water content in the presence of aspartic acid but they observed a longer ACC lifetime, which our results confirm. They argued that the mechanism by which additives stabilize ACC is mainly by inhibiting crystal nucleation and growth. The high amino acid concentrations used in our study and the minimal sorption of amino acids that we observed show that a large fraction of the added amino acids remain in solution and that these likely inhibit nucleation and crystal growth. Further support for this comes from calcite growth studies, where the effects of amino acids on bulk precipitation rates and calcite step velocities were quantified [14,15]. Specifically, a higher growth inhibition was observed for aspartic acid than for glycine because of

the extra carboxylic group and the particular conformation of ASP, which provides a better fit to the calcite surface sites [15] and thus likely blocks growth sites. Our current study supports and further confirms this interpretation. In solutions with 13 and 26 mM ASP, we see longer ACC lifetime (2 – 4 times longer than equivalent pure system) and at the highest ASP concentration (87 mM) we see a 6 minute induction time for crystal nucleation. Furthermore, calcite crystals with rounded edges formed in the presence of ASP, confirming its effect on growth. In contrast, in solutions with glycine, where the amino acid also sorbed to the ACC surface, the compound does not retard ACC crystallization as much (Fig. 1C) and ACC still forms at GLY concentrations of 87 mM. Finally, calcite formed in GLY had the same morphology as crystals grown in the pure system, indicating little if any effect of GLY on calcite growth (Fig 2C).

4. Summary

Results from this study show that the sorption of ASP and GLY both affect the crystallization kinetics of CaCO₃ biominerals that form in their presence, to a greater extent in solutions with ASP than GLY. This occurs through enhanced stabilization of the precursor ACC and thus an amino acid inhibition effect during the formation of the crystalline calcium carbonates. However, the ACC composition is little affected and thus any ASP or GLY associated with the ACC plays a minor role in the crystallization process. The stronger impact observed for ASP is explained by ASP binding more strongly to calcite surfaces than GLY, thereby blocking calcite nucleation more effectively. This resulted in calcite crystals with rounded edges whereas in the pure system or in the presence of GLY, crystals had smooth terraces and sharp edges.

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