Morphological templating of metastable calcium carbonates by the amino acid leucine

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Abstract. The in vitro precipitation of the metastable CaCO$_3$ phases aragonite and vaterite in the presence of leucine is investigated. Under normal conditions, the production of CaCO$_3$ via the hydrolysis of urea method favours the formation of regular needle-like aragonite crystals, with very minor quantities of vaterite and calcite. However in the presence of leucine, aragonite forms highly branched structures and the vaterite yield is increased, forming flower-like clusters composed of nano-thin sheets. Both the degree of aragonite branching and the occurrence, regularity of shape and number of vaterite “petals” increases with leucine concentration. The two phases exhibit different variations in their crystallographic parameters with increasing concentration, while the molecular structure appears unaffected.

1. Introduction
Advances in technology and science are often driven by the development of new materials, or by the discovery of novel properties of existing ones. The development of various ways of tailoring the structures of existing materials to unusual and special morphologies at the sub-micron and nano scale has been particularly productive [1]. Biomimetic synthesis, inspired by the remarkable range of morphologies exhibited by biominerals, is one such approach where large macromolecules are used to control growth morphology.

Calcium carbonate (CaCO$_3$) polymorphs are important in geological [2], environmental [3] and bio-sciences [4], as well as in numerous industrial applications, where control of polymorphism, morphology and crystal size distribution are important factors. CaCO$_3$ has three anhydrous polymorphs of increasing thermodynamic stability: vaterite (hexagonal), aragonite (orthorhombic) and calcite (rhombohedral). Aragonite particle size and shape depend strongly on preparation method and conditions, with direct inorganic precipitation from soluble carbonate and Ca-salts yielding irregular crystals [5] limiting their suitability for industrial application where high-aspect ratio CaCO$_3$ needle-like crystals are in much demand for improving the mechanical properties of polymer materials, or as a filler for paints, plastics, rubber, paper or foodstuffs [6]. It is also a potential biomedical material since it is denser than calcite and could be integrated, resolved, and replaced by bone [7]. Aragonite forms only under a narrow range of physio-chemical conditions and much effort has been given to developing reliable production methods [8] such as urea hydrolysis [9]. Vaterite also has potential practical applications due to high specific surface areas, high solubility, high dispersion and low density. However it is poorly understood structurally [10] and tends to form only small crystals. The high-volume commercial availability of amino acids offers a varied source of potential templating
macromolecules for industrial production and, as part of a study investigating the templating ability of various amino acids, we report here on the effect leucine has on the precipitation of metastable CaCO$_3$. Leucine is a neutral, essential $\alpha$-amino acid of the aliphatic group synthesized in plants and microorganisms and, as in nearly all $\alpha$-amino acids, exists in two optical isomers denoted as L-leucine (L-leu) or D-leucine (D-leu), which are mirror images of each other. Besides practical applications, amino acids of likely extraterrestrial origin are found in certain types of meteorite [19]; while understanding the localised interaction of amino acids with the surrounding environment may provide clues to the early conditions and evolution of biomineralising creatures and the early appearance in the fossil record of protective or skeletal

Figure 1. SEM micrographs of (a) aragonite grown in absence of amino acid; (b-e) aragonite grown in the presence of 2 g/L L-leu, 4 g/L L-leu, 6 g/L D-leu, 10 g/L L-leu; (f-j) vaterite in the presence of 2 g/L D-leu, 4 g/L L-leu, 4 g/L D-leu, 6 g/L D-leu, 10 g/L D-leu; Bottom right: molecular structure of L- and D-leucine molecules.
structures between the Ediacaran and Early Cambrian periods 525-542 Million years ago [20].

2. Experimental details
Analytical grade CaCl$_2$·H$_2$O (VWR International), urea ((NH$_2$)$_2$CO, Fischer Scientific) and leucine (HO$_2$CCH(NH$_2$)CH$_2$CH(CH$_3$)$_2$ L- and D- forms, Sigma Aldrich) were used to synthesize crystals of aragonite via direct precipitation in 50 ml deionised water by ageing solutions of CaCl$_2$ (0.25 mol dm$^{-3}$) and urea (0.75 mol dm$^{-3}$) at 90°C for 20 hours [9]. The precipitates were removed by filtration, rinsed with distilled water, dried overnight at 90°C and stored in a desiccator. The procedure was repeated with 0.1, 0.2, 0.3 and 0.5 g of L- and D-leu added to give 2, 4, 6 and 10 g/L concentrations. Precipitate morphologies were imaged by SEM (Hitachi TM1000) and characterised by confocal Raman spectroscopy (Horiba LabRam 800, 532 nm NdYAG laser, 600 line grating, 10x objective) and synchrotron X-ray powder diffraction (beamline I11, Diamond Light Source [16]) at 15 keV, with samples in 0.5 mm glass capillaries.

3. Results
Fig. 1 shows SEM images of the precipitates. Those grown without leucine (Fig. 1a) show the expected aragonite needle morphology [9], while increasing concentrations of leucine result in progressively split branching morphologies (Fig. 1b-e). For concentrations above 4 g/L the splitting occurs along the whole length of what would otherwise have been a single needle. Minor differences between L- and D-leu were apparent: L-leu precipitates showed a slightly

Figure 2. (a) Raman spectrum obtained from branched 4 g/L L-leu aragonite structure; (b) Raman spectrum obtained from 4 g/L D-leu vaterite flower. (c) X-ray powder diffraction pattern for CaCO$_3$ grown in presence of 4 g/L L-leu, showing the main aragonite (A) and vaterite (V) peak assignments (unmarked peaks are residual calcite phase).
Figure 3. Variation of measured crystallographic parameters of the precipitated metastable CaCO$_3$ phases as a function of leucine content: (a to c) aragonite (◇) lattice parameters; (d, e) vaterite (△) lattice parameters; (f) lattice strain for aragonite and vaterite; (g) crystallite size for aragonite and vaterite. For aragonite $a$, $b$, $c$ lattice parameters errors are less than $\pm 3 \times 10^{-5}$, $\pm 5 \times 10^{-5}$, $\pm 3 \times 10^{-5}$; For vaterite $a$ and $b$ are less than $\pm 8 \times 10^{-5}$ and $\pm 4 \times 10^{-4}$; errors in aragonite and vaterite strain are less than $\pm 1.3 \times 10^{-3}$ and $\pm 6 \times 10^{-3}$; errors in aragonite and vaterite crystallite sizes are $\pm 1.3$ and $\pm 10$ respectively across all data.

A higher degree of branching and blunt ends, while the D-leu templated structures retained sharper points. In the absence of amino acid, a small number of crystals with nano-thin irregular planar shapes were also formed. L- and D-leu increased the occurrence of these and, with increasing concentration, produced complex flower-like cluster structures, with large units on the outside and smaller ones concentrated towards the middle and top (Fig. 1f-j). Again only minor differences between L- and D-leu were observed: D-leu tended to yield flowers constructed from more regularly shaped objects at lower concentrations.

Separate Raman spectra were obtained for the branched and flower structures (Fig. 2a,b). The strongest carbonate band is the internal symmetric stretch at $\sim 1087$ cm$^{-1}$ and is
independent of polytype as it depends only on O atom motions [11]. External lattice modes due to translations of the ionic centres of gravity and carbonate ion librations (restricted rotations) appear in aragonite at 143, 181, 192, 206 cm$^{-1}$ along with a doublet at 702 and 706 cm$^{-1}$ [13]. The branched crystals show the 700 cm$^{-1}$ doublet, two strong features at 140 and 210 cm$^{-1}$ and a weaker one at 180 cm$^{-1}$, confirming their aragonite identity. In addition to the 1087 cm$^{-1}$ band, vaterite has an additional internal mode at $\sim$1074 cm$^{-1}$ with external modes at 118, 270, 303 and 750 cm$^{-1}$ [12] and confirms the flower structures as vaterite. Other than minor background differences, the spectra varied little between samples implying only growth and phase stabilisation, rather than molecular structure, are influenced by the amino acid.

From X-ray powder diffraction measurements (e.g. Fig. 2c) lattice parameters, crystallite size and microstructure strain for the two phases were obtained as a function of leucine concentration (Fig. 3) by whole pattern (150$^\circ$ 2$\theta$) LeBail profile refinement. For both phases, the lattice parameters (Fig. 3a-e) are affected by the presence of leucine, with the greatest effect appearing for concentrations of 2-4 g/L and is also reflected in the precipitated crystallite sizes (Fig. 3g). Above 4 g/L crystallite sizes are approximately constant. However the presence of the amino acid results in crystal structures with very different levels of internal strain (Fig. 3f). In Aragonite, the strain increases up to 4 g/L amino acid concentration, beyond which it declines, while vaterite precipitates with reduced strain at low concentrations, which increases above 4 g/L.

4. Discussion

Although the subject of many studies, the interaction between biomolecules, calcium ions and carbonate during morphological templating and preferential phase stabilization is unclear. In biological systems, crystal lattices can maintain their stability within the microenvironments formed by organic macromolecules [14] and small amounts of acidic macromolecules exist on nearly all organic/inorganic interfaces in biominaler structures (tooth, otolith, bone, pearl and mollusc shells etc.) [15]. Early investigations established that the concentration and the conformation of polypeptides rich in acidic amino acid entrapped onto the surface of organic matrices can control polymorph selection, while morphological templating can be achieved through the preferential bonding of macromolecules to specific atomic edge-step features on the surfaces of the precipitating crystals that offer the best geometric and chemical fit [18]. This in turn changes both the shape of the steps and the edge-step free energies. Changes in step shape directly modify the macroscopic crystal shape by inducing the formation of new crystal faces resulting in macroscopic morphological modifications [18].

The in vitro hydrolysis of urea occurs in two steps [9]. First, ammonium cyanate forms: CO(NH$_2$)$_2$ $\rightarrow$ NH$_3$ + HNCO $\rightarrow$ NH$_4^+$ + NCO$^-$ which then rapidly hydrolyzes: NCO$^-$ + OH$^-$ + H$_2$O $\rightarrow$ NH$_3$ + CO$_3^{2-}$. At elevated temperatures CO$_3^{2-}$ and HCO$_3^-$ ions are released and, by controlling the release rate, aragonite preferentially forms by homogeneous precipitation. Adding leucine to the urea/CaCl$_2$ solution provides additional bonding sites for Ca$^{2+}$ ions in the form of the amino acid carboxyl group and is clearly a factor in the stabilisation of the vaterite phase, possibly through charge balancing of the amino acid’s NH$_2$ group with the carbonate ions. The SEM results suggest preferential bonding of the amino acid via the Ca-carboxyl mechanism to the vaterite a-b plane, such that growth occurs on those faces perpendicular to the c-axis. At high concentrations coverage is presumably more extensive, with the macroscopic morphology (Fig. 1j) appearing to reflect the 6-fold symmetry of the hexagonal unit cell. There appears to be a strong relationship between morphology, crystallite size and internal strain in that for both phases the largest crystallites coincide with the highest strain. For aragonite, SEM imaging shows the greatest extent of both branching and branch size occurs for 4 g/L leucine concentration (Fig. 1c). At higher concentrations, the X-ray data suggests the internal strain is reduced by the precipitation of smaller crystallites and is evident in the SEM images (Fig.
The lattice parameters of both phases in the presence of the amino acid are distorted compared to those precipitated in its absence. Similar results with respect to geologic aragonite have been reported for biomineralised aragonites in mollusc shells and coral skeletons [17, 21] and attributed to the presence of macromolecules used to template structural growth. Altered strain states are thus likely to be a universal feature of macromolecule directed templating, biological or otherwise, and will alter the functional properties such as mechanical strength [22] or thermal stability [17] of the templated material under different conditions/environments and could, for example, be the underlying reason for differences in the aragonite-to-calcite transition temperature between aragonites of biogenic and non-biogenic origin [23].

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