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Journal of Inorganic Biochemistry 100 (2006) 1128-1138



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# Modelling calcium carbonate biomineralisation processes

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Received 28 September 2005; received in revised form 10 February 2006; accepted 13 February 2006 Available online 28 February 2006

### Abstract

The structure-directing influence of the organic dicarboxylates malonate, succinate, glutarate and adipate as templating species on the hydrothermal formation of CaCO<sub>3</sub> was investigated at different temperatures (60, 80, 90, 120, 150 and 190 °C) and with a range of molar ratios of  $[Ca^{2+}]/[templating species]$  (20, 14.3, 10, 7.7, 5, 1, 0.5 and 0.33). In the presence of the dicarboxylates, one, two or three polymorphs of CaCO<sub>3</sub> – calcite, aragonite and vaterite – could be formed, depending on the reaction conditions. In addition changes in crystal morphology were observed for the CaCO<sub>3</sub> polymorphs depending on the concentration of the template. In contrast, synthesis under ambient conditions of temperature and pressure resulted only in calcite formation, although template-dependent morphological changes were again observed. Crystalline products were all characterized by powder X-ray patterns and SEM (Scanning Electron Microscopy) micrographs. The ambient reactions with the chelating, dinucleating carboxylato ligands H<sub>3</sub>heidi and H<sub>5</sub>hpdta produce more profound changes in calcite formation of microtrumpets of constructed from bundles of nanocrystals of calcite is observed. The possible mode of action of these ligands on calcite formation is discussed in the context of known coordination chemistry with other metal ions. © 2006 Elsevier Inc. All rights reserved.

Keywords: Hydrothermal synthesis; CaCO<sub>3</sub>; Calcite; Aragonite; Vaterite; Polycarboxylates; Biomineralisation

### 1. Introduction

Some of the most remarkable and aesthetically pleasing structures seen in Nature are created via the process of biomineralisation. Biominerals are inorganic solid state structures formed by biological systems and in most cases are created to fulfil specific functions requiring hard and strong materials or else utilising a specific property of a solid state structure such as cooperative magnetic coupling. Examples of such structures include the beautiful and intricate solid state silica structures found in diatoms, the aligned single nanocrystals of magnetite used by magnetotactic bacteria to sense the Earth's magnetic field and our own teeth and bones made of hydroxyapatite and clearly fulfilling different roles in our own bodies [1,2].

In this paper we discuss some of our recent contributions to the specific field of calcium carbonate mineralisation, which we have approached from the point-of-view of coordination chemistry. In particular, we set out to see if by using some rather simple organic carboxylate-containing molecules it could be possible to shed some light on how Nature controls calcium carbonate formation. An important rationale behind this was the observation by a large number of workers in the area that carboxylates can influence calcium carbonate formation probably by blocking some of the carbonate sites in the lattice and modifying crystal growth [3–6]. In recognition of the observation that alongside the natural biominerals there was evidence that fairly complicated protein or polysaccharide molecules act as templating species, much previous work has concentrated on studying the effects on crystallisation of calcium carbonate in the presence of polypeptides and other large oligonuclear organics as well as using Langmuir-Blodgett approaches with long chain carboxylic acids [7]. However, as we report here, we find that it is not always

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<sup>0162-0134/\$ -</sup> see front matter @ 2006 Elsevier Inc. All rights reserved. doi:10.1016/j.jinorgbio.2006.02.012

necessary to have such complicated systems. Furthermore we have found that mildly hydrothermal conditions, sometimes where the temperature is still below that of the boiling point of water at normal pressure, offer a useful means of directing crystal phase. Here we carry out the crystallisations in a sealed acid digestion bomb at raised temperatures (between 60 and 190 °C) creating an autogenous pressure which can affect which phase is stable with respect to crystallisation. With the dual effect of the polycarboxylate additive there can be further control over phase and form as we describe below.

# 2. Choice of carboxylates

For our studies we chose to utilise two types of carboxylate-containing additives. Firstly, the simple aliphatic dicarboxylates of general formula  $(CH_2)_n(COOH)_2$  for n = 1-4 and secondly the two chelating polycarboxylates  $H_5$ hpdta, 1,3-diamino-2-hydroxypropane-N, N, N', N'-tetraacetic acid,  $(HOOCH_2)_2NCH_2(CH(OH))CH_2N (CH_2COOH)_2$ , and  $H_3$ heidi, N-2-hydroxyethyl-iminodiacetic acid,  $N(CH_2COOH)_2(CH_2CH_2OH)$ , which we have used previously in our work on hydrolytic iron(III) and aluminium(III) compounds.

### 3. Experimental

### 3.1. Synthetic methods

The polycarboxylates were used in mole ratios of  $Ca^{2+}$  to ligand of 25, 20, 14.3, 10, 7.7, 5, 1, 0.5 and 0.33 with the pH value of the polycarboxylates first adjusted to neutral values using 0.5 M sodium hydroxide solution.

In a typical reaction a solution of the polycarboxylate as the free acid formed in 5 ml water was neutralised carefully with 0.5 M NaOH solution, and CaCl<sub>2</sub> (0.25 mmol) was added with the appropriate molar ratio of polycarboxylate to calcium. To this solution was then added a solution of NaHCO<sub>3</sub> (0.50 mmol) in 5 ml water. The final pH was  $7.5 \pm 0.1$ . Alternatively, NaOH solution was not added, and then final pH was  $6.5 \pm 0.2$ . Hydrothermal reactions were carried out in parallel using Teflon-lined autoclaves with internal volume of 10 ml at temperatures between 60 and 190 °C under autogenous pressure. After 6 h reaction time the autoclaves were allowed to cool to 50 °C and maintained at that temperature for about 15 h before being allowed to cool slowly to ambient temperature over 3-4 h. Crystallisation experiments under ambient conditions were usually complete within 24 h.

### 3.2. Characterisation

The precipitates were isolated and examined through a light microscope and using scanning electron microscopy (SEM) to observe the sizes and shapes of the materials. The three crystalline phases of calcium carbonate can often be easily recognised from their distinctive shapes, but in all cases further phase identification was achieved using X-ray powder diffraction (XRD). This is especially important for cases where crystal growth was modified by the polycarboxylate. X-ray powder patterns were measured at ambient temperature on a Stoe STADI-P diffractometer using Co-K<sub> $\alpha$ 1</sub> radiation (Ge monochromator) and Debye–Scherrer geometry. The powder patterns were used to estimate the percentage composition of the precipitates by comparing them with known mixtures of various proportions of the component calcium carbonate phases.

# 3.3. $[Ca(Hheidi)] \cdot 3H_2O(1)$

In one case a new calcium coordination compound was isolated in the form of single crystals and the structure was established by single crystal X-ray diffraction. H<sub>3</sub>heidi (0.176 g, 1.0 mmol) in 5 ml of water was dissolved by dropwise addition of 0.5 N NaOH solution. CaCl<sub>2</sub> (0.294 g, 2.0 mmol) was added, the solution was neutralized to pH  $\approx$  7.0 by further addition of the NaOH solution with stirring over 30 min. Colourless needle-shaped crystals were formed after 2 weeks, yield 32%. Found: C, 26.91; H, 5.72; N, 5.07%; Calc. for C<sub>6</sub>H<sub>15</sub>CaNO<sub>8</sub>: C, 26.76; H, 5.62; N, 5.20%. IR v/cm<sup>-1</sup>: 3317 mbr, 2905 m, 2847 m, 1604 vs, 1448 m, 1401 s, 1338 m, 1325 mw, 1255 ms, 1228 w, 1160 m, 1129 m, 1078 ms, 1060 w, 1021 m, where v = very, s = strong, m = medium, w = weak, br = broad.

### 3.4. Crystallography

The single crystal structure of  $[Ca(Hheidi)] \cdot 3H_2O$  was measured at 200 K on a Bruker SMART Apex CCD diffractometer using graphite-monochromated Mo-K<sub>al</sub> radiation ( $\lambda = 0.71069$  Å). C<sub>6</sub>H<sub>15</sub>CaNO<sub>8</sub>; M (g mol<sup>-1</sup>) 269.27; Crystal size (mm)  $0.32 \times 0.08 \times 0.05$ ; monoclinic, space group  $P2_1/c$ ; a = 15.1467(16); b = 6.6046(7); c = 12.4821(13)Å;  $\beta = 108.673(2)^{\circ}$ ;  $V = 1183.0(2)Å^3$ ; Z = 4; F(000)568;  $D_c$  1.512 g cm<sup>-3</sup>;  $\mu$  (Mo K $\alpha$ )) 0.557 mm<sup>-1</sup>; 4664 reflections measured ( $R_{int}$  0.0302) of which 2272 independent and 2073 with  $I > 2\sigma(I)$ ;  $wR_2$  (all data) = 0.1534;  $R_1 = 0.0725$ ; largest peak/hole +0.47/-0.55 e Å<sup>-3</sup>. The structure was solved by direct methods and refined by full-matrix least-squares against  $F^2$  for all data using the SHELXTL software package [8]. All non-H atoms were refined anisotropically; organic H atoms were placed in calculated positions while the coordinates of O-H or water H atoms were refined.

Crystallographic data (excluding structure factors) for this structure have been deposited with the Cambridge Crystallographic Data Centre as Supplementary Publication No. CCDC 283406. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK: http:// www.ccdc.cam.ac.uk/perl/catreq/catreq.cgi, e-mail: data\_ request@ccdc.cam.ac.uk, or fax: +44 1223 336033.

# 4. Results

### 4.1. Background context

In general, biology tends to make use of available inorganic species alongside the familiar building blocks of the dominating "organic" part of Nature. In order to produce the solid state inorganic structures of biominerals, however, it is necessary to be able to control the solubility of the constituent ions so that the mineral is deposited in the right place and to be able to influence the growth of the mineral in terms of its shape and size in the final structure. There are two further important aspects to the process of biomineralisation. The first is that of control over mineral phase. For example, in the case of the biomineral systems we discuss here, which form in aquatic environments and are composed of calcium carbonate, there are three crystalline polymorphs (see Fig. 1 top) known from geology; calcite, aragonite and vaterite, plus an amorphous phase and it turns out that Nature uses the rather different characteristics of these to good effect by somehow selecting the phase required for the job in question. One of the most striking examples is furnished by aquatic creatures such as oysters, mussels and abalones which produce calcium carbonate shells where the outer part protecting the soft body of the mollusc from enemies is made of layers of calcite whilst the inner surface is fashioned out of shiny, smooth nanoscale aragonite, known as nacre, but more commonly familiar as mother-of-pearl (see Fig. 1 middle). We can note in passing one negative aspect of biological mineralisation which presents itself as the unwanted formation of a solid state phase such as the calcium carbonate deposit which forms around the irritating grain of sand acting as a nucleation point for the formation of a pearl in an oyster. In addition to trying to gain insights into how phase control can be achieved we also hope to discover how the crystallites in, for example, the coccolithophoridae, can be organised into such intricate shapes (Fig. 1, middle). There has been much recent interest in the marine examples of these algae whose amazing calcium carbonate structures have only been "visible" since the advent of electron microscopy. One motivation for investigating these is that they "bloom" under certain favourable aquatic conditions which are thought to involve high light intensities and low phosphate levels with carbon dioxide levels probably also playing a role. For example, in the cretaceous era when there were shallow warm seas, such as those around the area which is now the south coast of Britain, algal blooms achieved vast proportions and the calcite coccoliths of these creatures went on to form the famous "White Cliffs of Dover" through sedimentation processes. Although the idea that the coccolithophoridae might provide a useful means of removing CO<sub>2</sub> from the atmosphere in these times of excess greenhouse gases is attractive, the processes of CO<sub>2</sub> dissolution are very complicated with

dynamic equilibria in operation as well as being highly and pH-sensitive. In fact, in the final stage of the process of calcium carbonate formation two moles of hydrogen carbonate are converted to one of carbonate and one of CO<sub>2</sub> suggesting perhaps that calcium carbonate formation by these creatures is not necessarily a good thing. However, the organisms also fix gaseous carbon dioxide via photosynthesis and the verdict must be left open on this aspect. One thing that is certain is that when the algal blooms occur (see Fig. 1 bottom), not only are they often of vast proportions with areas of up to 100,000 km<sup>2</sup>, but also there is a significant effect on the reflectivity of the ocean which has important consequences on global warming [9,10]. This second remarkable aspect of biomineralisation of the organisation of individual crystalline building blocks on one length scale to give structures with a larger length scale has been termed "crystal tectonics" and can be visualised in terms of the process of building a house from bricks and mortar [11–13], but at the length scales in biomineral structures the agent building the structure is far from obvious.

# 4.2. Calcium carbonate formation with polycarboxylates

In these experiments we used an approach similar to the one previously employed by us in hydrolytic reactions (see discussion), but with the difference that the final mineral phase the reaction is heading towards is now a carbonate rather than an oxyhydroxide or oxide. We can summarise the reactions thus:

$$xCa^{2+}(aq) + yHCO_{3}^{2-}(aq) + n(polycarboxylate)$$
  

$$\rightarrow \{Ca_{x}(polycarboxylate)_{n}(CO_{3})_{y}\} + H^{+} \rightarrow xCaCO_{3}(s)$$

In fact, we have so far been unsuccessful in characterising an intermediate phase corresponding to  $\{Ca_x(polycarboxy$  $late)_n(CO_3)_y\}$  but we can also note the role played by the carboxylate groups in mimicking carbonates.

# 4.2.1. Crystallisation under ambient conditions of temperature and pressure

Under ambient conditions in the absence of any templating species only calcite crystals were formed as was expected. This was also found to be the case when malonate, adipate or succinate was added, even with high dicarboxylate concentrations. In the case of glutarate small amounts of vaterite were formed with high glutarate concentrations ([Ca<sup>2+</sup>]/[glutarate] mole ratio 5 or less). However, the calcite crystal morphology changed in the presence of the dicarboxylate. In place of the familiar rhombohedral calcite crystals (similar to those in Fig. 1), with increasing dicarboxylate concentration spindle-shaped crystals of calcite were obtained. These crystals were elongated along the c-axis with the {104} faces dominant. Similar morphologies have been previously reported from ambient syntheses in the presence of dicarboxylate species [3].



Fig. 1. (Top) The crystalline polymorphs of calcium carbonate: (a) calcite, (b) aragonite, (c) vaterite. (Middle) Examples of calcium carbonate structures from biology: (d) Cross-section through bivalve shell. The upper part is the tough calcite of the outer shell, the lower the shiny nacreous layer. (e) The coccolithophore *discosphaera tubifera*. (Bottom) Satellite shots of an algal bloom approaching the mouth of the English Channel between the Cornish Peninsula and Brittany (f) and a bloom off the Cornish coast (g).

### 4.2.2. Hydrothermal crystallization

Calcite crystals of typical rhombohedral morphology were obtained through hydrothermal crystallization at 60–190 °C in the absence of a templating species. Examples of such calcite crystals produced at 150 °C are shown in Fig. 2(a). Crystals were found with sizes ranging from 20 to 80  $\mu$ m. On addition of malonate, succinate, glutarate or adipate as templating species, the three polymorphs of CaCO<sub>3</sub> – calcite, aragonite and vaterite – were obtained, either as pure phases or as mixtures of varying compositions. Depending on the reactions conditions, novel crystal morphologies were observed. 4.2.2.1. Effect of malonate. When  $CaCO_3$  was synthesized under hydrothermal conditions in the presence of malonate at pH 7.5, a morphological change was observed in the calcite crystals obtained. The elongated rhombohedral crystals formed under ambient conditions become progressively truncated towards the {001} face, eventually resulting in hexagonal tabular crystals (Fig. 2(b)). This change in morphology became more dominant with higher malonate concentrations and also as the hydrothermal temperature was increased from 60 to 150 °C.

When the synthesis was carried out at pH 6.5 in the same range of hydrothermal temperatures, however, mix-



Fig. 2. Examples of the phases and morphologies of calcium carbonate crystals formed under the conditions given in the text: (a) Calcite crystals produced hydrothermally; (b) hydrothermally produced calcite in the presence of malonate; (c) vaterite crystals formed hydrothermally at 60 °C in presence of malonate at pH 6.5; (d) and (e) calcite formed through hydrothermal reactions at 150 °C in the presence of succinate; (f) barrel-shaped vaterite forms predominantly under hydrothermal conditions at 150 °C at higher glutarate concentrations whereas (g) at lower concentrations with  $[Ca^{2+}]/[glutarate] = 0.5$  vaterite balls, aragonite needles and calcite are observed together; (h) hexagonal rods of aragonite alongside rhombic calcite formed at 90 °C with pH 7.5 and  $[Ca^{2+}]/[adipate] = 1.0$ .

tures of calcite and vaterite were observed. The proportion of vaterite formed increased with higher concentrations of malonate, becoming the major product. Fig. 2(c) shows the disk-shaped aggregates of vaterite crystals, with diameter ca. 20 µm, formed at 80 °C with  $Ca^{2+}$ : malonate = 5.

4.2.2.2. Effect of succinate. When succinate was used as the templating agent at either pH 6.5 or 7.5, then similar calcite crystals were obtained to those formed with malonate at pH 7.5 (Fig. 2(d)–(e)). In contrast to the results with malonate, no vaterite was formed at pH 7.5.

4.2.2.3. Effect of glutarate. At pH 7.5, calcite crystals were obtained with low glutarate concentrations ( $[Ca^{2+}]/[glut$ arate = 20) at hydrothermal temperatures from 60 to 120 °C. As the glutarate concentration was increased  $([Ca^{2+}]/[glutarate] = 10, 7.7 and 5)$ , mixtures of calcite and aragonite were found over the temperature range 80-190 °C. Hexagonal plate-like crystals of calcite and radiating needles of aragonite formed at 150 °C with  $[Ca^{2+}]/[glutarate] = 5$ . At high glutarate concentrations  $([Ca^{2+}]/[glutarate] = 1.0, 0.5, 0.33)$  and temperatures up to 120 °C, however, vaterite becomes the major product and was found to have a barrel-shaped morphology under these conditions (Fig. 2(f)). Fig. 3(a) shows the powder X-ray pattern of the product formed with  $[Ca^{2+}]/[glutarate] = 1.0$  at 80 °C, with strong peaks from both vaterite and calcite, together with traces of aragonite. At higher temperatures (150 and 190 °C) the yields of calcite and aragonite became more significant, and mixtures of all three polymorphs could also be obtained with intermediate glutarate concentrations at all temperatures. Fig. 2(g) shows a rhombic crystal of calcite, ballshaped aggregates of vaterite and needles of aragonite formed at 150 °C with  $[Ca^{2+}]/[glutarate] = 0.5$ . The diameters of the ball-shaped polycrystalline aggregates of ranged from 10 to 100 µm. Similar results were obtained at pH 6.5, although the proportion of vaterite formed was generally rather higher. Fig. 3(b) shows the X-ray powder pattern corresponding to the mixture shown in Fig. 2(g), which confirms that aragonite is the major (ca. 70%) product, and that calcite is the only other product, with no vaterite detectable from these conditions.

4.2.2.4. Effect of adipate. The use of adipate as the templating species strongly favours the formation of aragonite under most conditions. Fig. 2(h) shows the mixture of aragonite and calcite crystals formed at 90 °C with pH 7.5 and  $[Ca^{2+}]/[adipate] = 1.0$ . The length of the hexagonal needles of aragonite varied from 50 to 100 µm, while the rhombs or truncated rhombs of calcite ranged from 15 to 35 um. Aragonite formation increases on raising the hydrothermal temperature from 80 to 190 °C and also as  $[Ca^{2+}]/[adipate]$ decreases from 20 to 0.33. Fig. 3(b) shows the X-ray powder pattern corresponding of the mixture shown in Fig. 2(h), which confirms that aragonite is the major (ca. 70%) product, and that calcite is the only other product, with no vaterite detectable from these conditions. This is shown graphically in Fig. 4(a), where the proportion of calcite formed is plotted against hydrothermal temperature for various values of  $[Ca^{2+}]/[adipate]$ . Aragonite formation was even more strongly favoured at pH 6.5. Fig. 4(b) shows the proportion of calcite formed plotted against hydrothermal temperature at pH 6.5 and 7.5, with  $[Ca^{2+}]/[adi-$ [pate] = 7.7 in both cases.



Fig. 3. Representative X-ray powder patterns of the calcium carbonate mixtures formed hydrothermally: (a) with  $[Ca^{2+}]/[glutarate] = 1.0$  at 80 °C and (b) with  $[Ca^{2+}]/[adipate] = 1.0$  at 90 °C with pH 7.5 corresponding to the mixture shown in Fig. 2(h) (A, aragonite; C, calcite; V, vaterite).



Fig. 4. Graphical representations of proportion of calcite formed plotted against hydrothermal temperature for various ratios of  $[Ca^{2+}]/[adipate]$  at (a) pH 7.5 and (b) at pH 6.5 and 7.5, with  $[Ca^{2+}]/[adipate] = 7.7$ .

### 4.3. Calcium complex with $H_3$ heidi

Reaction of CaCl<sub>2</sub> with H<sub>3</sub>heidi (*N*-2-hydroxyethyliminodiacetic acid) in aqueous solution, with NaOH or KOH as base, yielded the complex  $[Ca(Hheidi)] \cdot 3H_2O$ (1) in good to moderate yield.

The structure of 1 is shown in Fig. 5. Each (Hheidi)<sup>2–</sup> ligand (the hydroxyl oxygen is still protonated) chelates a calcium cation through the nitrogen, two carboxylate oxygens, O(1) and O(3), and the hydroxyethyl oxygen O(5). O(1) and its inversion-related counterpart O(1a) then form a pair of monoatomic bridges to Ca(1a) at  $\{-x, -y, -z+1\}$ . The second carboxylate group bridges between three calcium centres. O(3) bridges from Ca(1) to Ca(1b) at  $\{-x, y+1/2, -z+3/2\}$ , while O(4) coordinates to Ca(1c) at  $\{x, y+1, z\}$ , and this carboxylate group thus forms a *syn,syn* bridge between Ca(1b) and Ca(1c). These bridging motifs for the carboxylates have parallels with the carbonate bridging observed in the polymorphs of calcium carbonate.

These linkages result in the build-up of two-dimensional sheets in the crystal, which lie parallel to the 100 crystal plane. These sheets are then held together by hydrogenbonding involving the lattice waters and carboxylate oxygens. The calcium cations within a sheet form a slightly corrugated *quasi*-hexagonal array, as shown in Fig. 5, with Ca...Ca distances of 4.115 and 4.243 Å.

# 4.4. Calcium carbonate formation with $H_3$ heidi and $H_5$ hpdta

Introducing a chelating effect into the polycarboxylate produces some surprising results. As we noted above from the results obtained with the iron systems, dicarboxylates can effectively replace sites from simple oxygen donors such as hydroxides as seen in the defect brucite structure observed for  $[Fe_2(OH)(ox)]$  [14,15] (where ox = oxalate) and this would seem to be the case for the calcium systems where a carboxylate can replace the oxygen donors of a carbonate and act as a growth modifier. When we use a chelating and potentially dinucleating ligand such as H<sub>3</sub>heidi or H<sub>5</sub>hpdta the effects on calcium carbonate formation are more dramatic and especially so under ambient conditions. As indicated above, with a relatively large amount of H<sub>3</sub>heidi present it is possible to obtain single crystals of a calcium coordination network with the doubly deprotonated ligand. At lower concentrations of the polycarboxylate we find that calcite formation is strongly influenced (S.B. Mukkamala and A.K. Powell, manuscript submitted) and crystal shapes resembling otoliths (Fig. 6) as found in the balance organ in human ears are formed. This intriguing result could be of value in understanding more about the distressing conditions resulting from improper growth of these important structures which is the cause of a number of medical conditions including deafness and has been extensively studied in the case of zebrafish [16].

With the polycarboxylate 1,3-diamino-2-hydroxypropane-N, N, N', N'-tetraacetic acid, H<sub>5</sub>hpdta, the situation is even more intriguing. We find that once we have only small amounts of the polycarboxylate present microtrumpets of calcite are produced [17] which are composed of what appear to be long nanocrystals of calcium carbonate (Fig. 7). These structures can be compared with the shapes found on the coccolithophore *discosphaera tubifera* (Fig. 1 middle). This organisation of nanoscale crystals into a microscale structure is remarkable for two reasons. Firstly, the process by which this proceeds mimics the way in which biomineralisation can produce organised structures with



Fig. 5. (Above) Coordination geometry in  $[Ca(Hheidi)] \cdot 3H_2O$  (1); (Below) structure of the 2-D sheets in 1 (the Ca centres are joined by pink dotted lines to emphasise the hexagonal structure).

different length scales. Secondly, the polycarboxylate we have used is really rather a simple chemical species quite unlike the complicated polysaccharide implied in the production of coccoliths. These facts lead us to question how the process might work. As we have already reported, we can follow the process, which produces the trumpet shapes within 24 h, by isolating intermediate precipitates and in this way observe the growth of the trumpets. We found that the initial precipitates are composed of nanostructured calcium carbonate plaques and as yet we do not know which of the possible phases these belong to. From these plaques it seems that bobbles form and from these nano-



Fig. 6. The calcite crystal morphology observed when  $H_3$  heidi is present under ambient conditions.



Fig. 7. The final form of a calcite microtrumpet formed in the presence of  $H_5$ hpdta.

bobbles the next structure we observe is like a conical hat shape which then evolves into the final predominant form of the precipitate as nanostructured microtrumpets.

Perhaps the structures we observe for the calcium/Hheidi<sup>2-</sup> coordination network and the iron(III) and aluminium(III) systems with hpdta<sup>5-</sup> we have reported previously [18–25] can offer some clues. In compound **1** the calcium has a coordination number of 7 and with its relatively large ionic radius we can expect with a good degree of confidence that the coordination number will usually be higher than for Fe(III) and Al(III). As we have seen for the iron and aluminium systems where the coordination number is 6 we have the situation where, for example in the Fe(III) dimers which form with hpdta<sup>5-</sup>, there are two iron centres coordinated by one hpdta<sup>5-</sup> ligand with the deprotonated alcohol function bridging between the two and with the coordination spheres for each metal centre completed by the rest of the donors from the ligand plus two further ligands such as water. For the iron and aluminium systems these water ligands are ideally placed to allow hydrolytic reactions to produce the species we mentioned above.

What might the scenario be for calcium? Firstly we can see from the structure of 1 that  $Ca^{2+}$  is not a strong enough acid to deprotonate the alcohol of the H<sub>3</sub>heidi ligand under these conditions. But we can also note that symmetrical, potentially dinucleating ligands such as H<sub>5</sub>hpdta tend to lose the alcohol proton and form dinuclear compounds with most metal ions. Although there is not a direct example of this for calcium we have found similar results in the case of  $Cu^{2+}$  systems with related dinucleating ligands [26] and we can expect that for the H<sub>5</sub>hpdta ligand calcium binding is likely to involve this dinucleating function of the ligand. In this case we can also expect that the larger ionic radius and coordination number of the calcium ion to lead to a situation where the available coordination sites on the calcium ion are greater in number than for the iron and aluminium cases and that the fairly flexible  $hpdta^{5-}$ ligand is thus effectively splayed out, perhaps explaining how the growth modification of the calcite can lead to the bent nanocrystals seen in the trumpet structures. Even if this conjecture proves to be correct, we still need to discover what it is that enables these long nano-cross-sectioned crystals to be organised into the trumpet shapes. That surely is the most remarkable thing about this seemingly simple system.

### 5. Discussion

# 5.1. Synthetic strategies and previous observations

The underlying synthetic concept used above is adapted from our previous work on directing crystal growth and morphology in iron and aluminium hydroxide based systems [18–25] where we use polycarboxylates to influence the outcome of hydrolytic reactions according to:

$$\begin{split} \mathbf{M}_{(\mathrm{aq})}^{a+} &+ \mathrm{polycarboxylate}_{(\mathrm{aq})}^{m-} + \mathrm{base}_{(\mathrm{aq})} \\ &\rightarrow \{M_x \mathrm{polycarboxylate})_n(O)_y(\mathrm{OH})_z(\mathrm{H}_2\mathrm{O})_p\}_{(\mathrm{aq})}^{\pm b} \\ &\rightarrow \mathrm{M}_2\mathrm{O}_a(\mathrm{s}) \end{split}$$

There we found that the correct choice of reaction conditions allows us to stabilise fragments of oxyhydroxide and hydroxide mineral phases of general formula  $\{M_x \text{poly-} \text{carboxylate}\}_n(O)_y(OH)_z(H_2O)_p\}^{\pm b}$  or else to modify the resulting oxide/oxyhydroxide phase of general formula  $M_2O_a$ . The former case gives rise to the possibility of observing intermediate crystal structures containing the templating polycarboxylate incorporated into defect hydroxide mineral structures. We found that using hydrothermal conditions gives access to mineral species which are rarely produced under normal conditions in a purely geological context although they are observed in biological systems. For example, we were able to isolate the compound  $Fe_2^{II}ox(OH)_2$  which is an intermediate phase equivalent to a defect brucite (M(OH)<sub>2</sub>) with pairs of hydroxide sites replaced by oxalate oxygens. We found that the final product of such a synthesis is magnetite [14,15]. Although geologically formed magnetite usually only results under a very narrow set of reaction conditions Nature is very adept at producing uniform nanocrystals of this magnetic mineral under ambient, neutral conditions for use as sensors for the Earth's magnetic field for orientation and navigational purposes. The utility of hydrothermal synthesis as a means of modelling biomineralisation processes where metastable phases occur has been referred to above. Thus, although the mineral magnetite is not the thermodynamically most stable product in biogenic or geological iron oxide formation the phase can be produced either through biogenic control or else by adjusting the parameters such as temperature and pressure in order to stabilise this phase. In this way, hydrothermal conditions can be used to assist the biomimetic chemist in achieving what biology usually does under less extreme conditions.

# 5.2. Fe(III) with $H_3$ heidi

When we use the chelating and dinucleating polycarboxylate heidi<sup>3-</sup> we can crystallise an  $Fe_{19}^{III}$  aggregate (Fig. 4) with an inorganic a core again corresponding to the M(OH)<sub>2</sub> brucite structure [18,19]. This turns out to be an excellent miniature model for loaded ferritin [27,28]. One thing which seems to be a useful synthetic aid is the dinucleating ability of the alcohol arm of the heidi<sup>3-</sup> ligand. In fact, this is merely a further manifestation of the ability of a good acid such as Fe(III) to deprotonate –OH groups which then naturally tend to form bridges between metal centres as observed in the hydroxide minerals.

# 5.3. Fe(III) and Al(III) with $H_5$ hpdta

Furthermore, we found that the dinucleating ligand hpdta<sup>5-</sup>, which can be regarded as a derivative of EDTA or, indeed, as akin to two heidi<sup>3-</sup> ligands fused together at the alcohol function, provides a useful means of presenting two hydrolytically active metal centres able to interact with a forming hydroxide mineral structure. The dinucleating ability of this carboxylate is seen in the myriad structures which can be obtained with Fe(III) which all have the dinuclear {Fe<sub>2</sub>(hpdta)(H<sub>2</sub>O)<sub>4</sub>}<sup>+</sup> unit as a structural element (see Fig. 8) [29,30].

In the case of the Al(III) ion, which we find often mimics Fe(III) systems [23–25], it was also possible to obtain an  $Al_{15}$  aggregate with this ligand [22] again with the brucite-type core seen in compounds such as the  $Fe_{19}$  aggregate mentioned above. This aggregate can be formally envisaged as an  $Al_7$  hydroxide unit encapsulated by four dinuclear structural units (Fig. 8) analogous to the dimer seen in the Fe(III) system.



Fig. 8. The  $Fe_{19}$  aggregate formed with heidi (top left); the  $Al_{15}$  aggregate formed with hpdta (top right) and the dinuclear building block shown for Fe(III) (bottom).

In the approach using ambient conditions we rely on reaching a situation where the solubility product of the material results in precipitation. In fact, we hope that the processes of nucleation and crystal growth can be steered in the direction of crystalline phases which can be observed and characterised so that we can truly gain some insights into how the biological system might be controlling the form and function of biominerals.

The results of the survey of the influence of the various polycarboxylates on the formation of calcium carbonate showed some interesting trends and surprising results.

# 6. Conclusions and outlook

Although in natural systems biomineral phase, shape and function are often controlled by rather complicated chemical species, we have found that under certain synthetic conditions it is relatively easy to exercise quite a large degree of control over calcium carbonate phase and morphology. Using simple dicarboxylates a variety of crystal shapes and phases could be produced when hydrothermal conditions were employed. The hydrothermal synthesis thus offers a means of stabilising phases of calcium carbonate other than calcite and in conjunction with the dicarboxylate can produce a variety of unusual shapes. When we use chelating and dinucleating polycarboxylates we find that ambient conditions can allow us to isolate unusual forms of calcite including remarkable trumpet shaped structures which are composed of long nano-cross-sectioned crystals formed into microscale trumpets. This represents a further dimension in modelling biomineralisation, namely that of crystal tectonics in which crystallites of one length scale are used to build structures of another length scale. We are currently following up this work with further studies on all of the systems discussed here with a view to learning more about the mechanisms in operation as well as the optimum way to control the formation of calcium carbonate. This research could be important for a number of different areas including control of scale formation and understanding more about the cycling of carbon dioxide and carbonate via aquatic systems.

# Acknowledgements

This work was supported by the DFG SPP 1117 on Biomineralisation and we thank P. Pfundstein for assistance with the SEM measurements.

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