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Monohydrocalcite and Its Relationship to Hydrated Amorphous Calcium Carbonate in Biominerals

Markus Neumann[a] **and Matthias Epple***[a]

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Calcium carbonate monohydrate (monohydrocalcite) is prepared and structurally characterised by X-ray diffraction, X-ray absorption spectroscopy (EXAFS), thermal analysis, IR spectroscopy, and scanning electron microscopy. It is compared to hydrated phases of amorphous calcium carbonate (ACC) that have frequently been observed in biomineralisation. The crystal water is more strongly bound than in hydrated ACC phases, and monohydrocalcite can be distinguished from ACC by its IR spectrum. The EXAFS results suggest that monohydrocalcite is a suitable model for some biogenic and synthetic ACC phases, although not for all of them.

Introduction

Monohydrocalcite, $CaCO_3{\cdot}H_2O$, was first observed in 1930 during the dehydration of ikaite ($CaCO_3{\cdot}6H_2O$).[1] It was first observed in nature in 1959 in marine sediments,[2] and the first synthetic preparation was accomplished by Brooks et al. in 1950.[3] Under ambient conditions, this phase is metastable compared to the water-free calcium carbonate modifications calcite and aragonite, and upon dehydration, or at higher temperature, it transforms into these phases. Consequently, its solubility is higher than that of the water-free modifications (calcite, aragonite, vaterite).[4] Magnesium is generally incorporated into this phase in small quantities, therefore it is assumed that magnesium plays an important role during the crystallisation.[5–7] In nature, it is formed preferably in magnesium-rich marine locations.[8] The single crystal structure with trigonal symmetry (including a superstructure) was determined by Effenberger in 1981.[9]

Many living systems use inorganic minerals, for example for protection (shell, exoskeleton), as tools (teeth), for defence (spicule) or for internal stabilisation (bones forming an endoskeleton).[10–16] Calcium carbonate is the most prominent biomineral and is the main constituent of mollusc shells, crustacean cuticles, corals, skeletons of protozoa, etc.[17] In these cases, very delicate and often exceedingly mechanically stable structures are built by these organisms which (so far) cannot be mimicked in the laboratory. An exact control over the pathway of crystallisation lies at the centre of this process: There is a finely tuned interplay between the crystallising inorganic mineral and bio-organic compounds that control the crystallisation, both at the level of nucleation as well as during crystal growth.[10–17] The formation of mesocrystals has been proposed as a mechanism of how nanocrystals assemble into macroscopic crystals and aggregates.[18,19] In the case of calcium carbonate, there is increasing evidence that amorphous calcium carbonate (ACC) is first formed as a solid phase which then undergoes rearrangement/recrystallisation to one of the crystalline phases of calcium carbonate (calcite, aragonite and, much less frequently, vaterite). This is supported by studies of the mineral phase in developing organisms[20–32] and also by studies in vitro,[33–36] where ACC has been prepared synthetically. The role of ACC in biology was recently reviewed by Addadi et al.,[27] Meldrum,[17] and Weiner et al.[31] The thermodynamic[37] and structural[36] properties of synthetic ACC have been discussed by Günther et al.

When biogenic ACC is studied it usually contains some water, which suggests a hydrate-like structure. However, a distinction must be made between stable (permanent) forms of ACC and transient forms of ACC which transform into a crystalline phase. Addadi, Raz and Weiner have suggested that the stable forms are hydrated whereas the transient forms are not.[27] A water content of 15.7 wt.-% has been determined thermogravimetrically for hydrated ACC in the spicules of *Pyura pachydermatina*, which compares well to the stoichiometric formula for monohydrocalcite ($CaCO_3{\cdot}H_2O$).[21] X-ray absorption spectroscopy (EXAFS) gave the best fit to the crystal structure of monohydrocalcite.[21] The monohydrocalcite EXAFS structure has also been found for ACC in cystoliths of *Ficus microcarpa* and *Ficus retusa*.[24]

We note here that all structural and thermal investigations that have suggested a similarity of hydrated ACC to monohydrocalcite refer only to literature data for monohydrocalcite, that is they use the crystal structure of monohy-

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[a] Inorganic Chemistry, University of Duisburg-Essen,
Universitätsstrasse 5–7, 45117 Essen, Germany
Fax: +49-201-183-2621
E-mail: matthias.epple@uni-due.de

drocalcite for EXAFS fits. Monohydrocalcite itself has never been studied by EXAFS spectroscopy. We therefore considered it appropriate to prepare this phase in pure form, to characterise it by modern methods and compare it to biogenic ACC.

Result and Discussion

Monohydrocalcite was synthesised by adding potassium carbonate to artificial seawater. It was clearly identified by X-ray diffractometry in a phase-pure form (Figure 1). A sample stored for three months at room temperature in a closed vial showed the same diffractogram, which means that monohydrocalcite is completely stable over that period and does not lose its water of crystallisation.

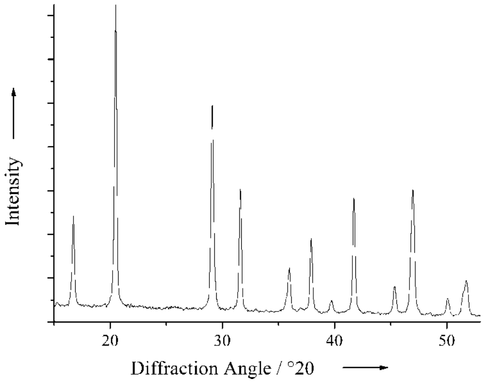

Figure 1. X-ray powder diffraction pattern of phase-pure monohydrocalcite.

The composition was studied by thermogravimetry, coupled with IR spectroscopic analysis of the released gases (Figure 2). A mass loss of 14.92 wt.-% from 140 to 205 °C was detected, which corresponds to the release of crystal water (theoretical value: 15.25 wt.-%). At higher temperatures (550 to 800 °C), decarboxylation occurred (observed mass loss: 36.44 wt.-%; theoretical value: 37.26 wt.-%). Released gaseous water and carbon dioxide, respectively, were detected in the connected IR spectrometer at the appropriate temperatures.

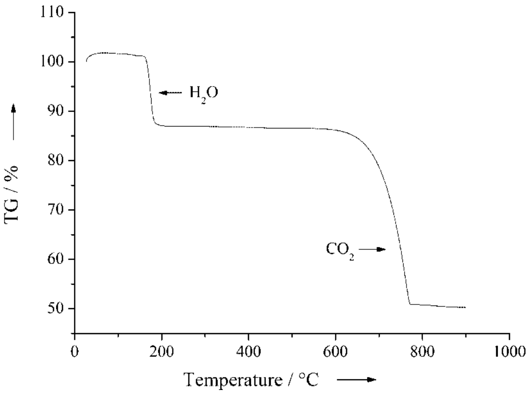

Figure 2. Thermogravimetric study of monohydrocalcite in a dynamic nitrogen atmosphere, showing first the dehydration and then the decarboxylation.

Synthetically prepared ACC has a water content of about 7.5 wt.-% (close to a hemihydrate), and about 1.5 wt.-% is released from 50–100 °C (loosely bound water) and about 6 wt.-% from 100–250 °C (structural water).[36] ACC from *Pyura pachydermatina* contains 15.7 wt.-% water (in good agreement with a monohydrate), which is released in the temperature range 20–200 °C.[21] We note that monohydrocalcite contains more strongly bound crystal water than ACC, a fact which is probably due to its higher crystallinity and its higher lattice energy.

Monohydrocalcite forms crystalline calcite after thermally induced loss of crystal water, as shown by X-ray powder diffraction (Figure 3). The fact that biogenic ACC can transform into calcite,[22,30,38] aragonite[20,26] and possibly vaterite[20] in vivo shows that there is a clear difference between a thermally induced dehydration and a biologically induced transformation at room temperature.

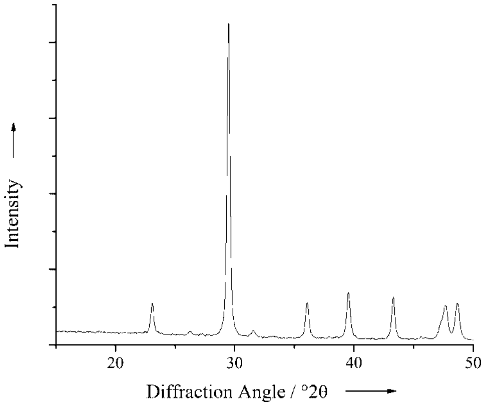

Figure 3. The X-ray powder diffractogram of monohydrocalcite after thermal dehydration (185 °C) shows only crystalline calcite.

The DSC curve shows an endothermal peak at 171.4 °C corresponding to the dehydration (Figure 4). The associated enthalpy change is +49.1 kJ mol^{-1}. Taking into account the enthalpy of evaporation of water [$\Delta_{vap}H_{373}°(H_2O)$ = 40.66 kJ mol^{-1}], the binding enthalpy of the water molecule is of the order of 8.5 kJ mol^{-1}.

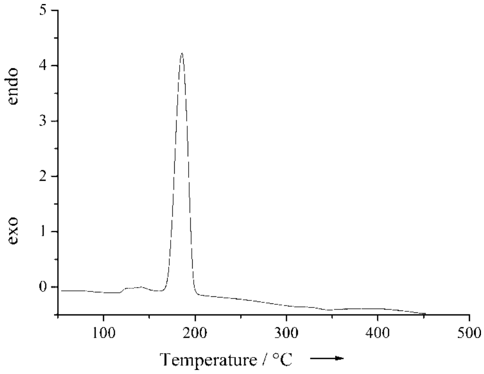

Figure 4. DSC curve of monohydrocalcite showing the endothermal peak associated with the dehydration. This is preceded by a broad endothermal peak which may be due to the release of water from disordered near-surface regions.

Monohydrocalcite was also characterised by IR spectroscopy (Figure 5). The asymmetric C–O stretching vibration is split (1408 and 1487 cm^{-1}). This is also observed for aragonite and vaterite, whereas calcite shows no splitting. In ACC from sternal deposits of the woodlouse *Porcellio scaber* we found an IR band at $\tilde{\nu}$ = 860 cm^{-1} (CO_3^{2-}; A_{2u}).[28] The following values have been reported for this band in the literature: aragonite (860 cm^{-1}), calcite (876 cm^{-1}), and vaterite (877 cm^{-1}).[39] For biogenic ACC, a value of 866 cm^{-1} has been reported.[27] Monohydrocalcite shows bands at 590 (lattice), 700/727 (ν_4 of carbonate), 766 (lattice), 873 (ν_2 of carbonate), 1068 (ν_1 of carbonate), 1408/1487 (ν_3 of carbonate), 1700 (H_2O deformation), and 3235 cm^{-1} (O–H stretch), in good agreement with the literature.[8,27,40,41] We therefore conclude that monohydrocalcite and ACC can be distinguished by IR spectroscopy by observing the carbonate band at around 870 cm^{-1}.

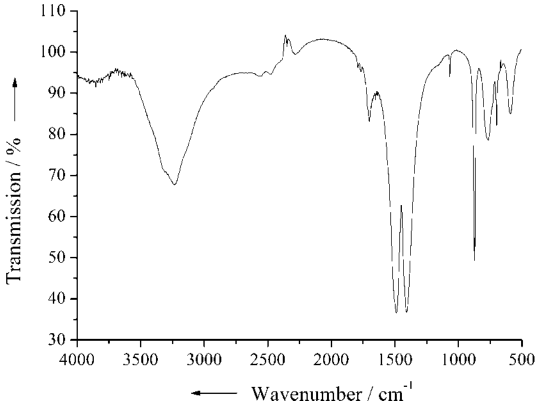

Figure 5. IR spectrum of monohydrocalcite.

The internal structure of monohydrocalcite was also studied by EXAFS, which examines the short-range order around Ca. Figure 6 shows the Fourier transform magnitude and the raw EXAFS data.

EXAFS is a versatile tool, especially for the analysis of amorphous materials which have short-range order but no long-range order. Investigations of amorphous calcium carbonates of different origin have shown that they differ in their short-range order.[27,36,43] The most stable forms of ACC contain one molecule of water per carbonate molecule, therefore monohydrocalcite has been suggested to be an appropriate model for hydrated ACC.[24,27] In the case of the ascidian *Pyura pachydermatina*, the best fit was actually obtained using monohydrocalcite as a model (see data in Table 1).[24] It is notable that no fit of the Ca–Ca coordination at around 4 Å was performed,[21] which shows that the disorder in ACC is much higher than in crystalline monohydrocalcite. The same situation has been observed for synthetic ACC, where only the first shell (Ca–O) could be fitted to the experimental data.[36] The transient amorphous calcium carbonate in the sternal deposits of the crustacean *Porcellio scaber* resembles either vaterite or monohydrocalcite.[28] Taylor et al. were the first to use EXAFS for the investigation of amorphous calcium carbonate from cystoliths of *Ficus retusa*. They showed that in the first coordination shell Ca is surrounded by eight O atoms, which is also the case for monohydrocalcite, and related it to ikaite ($CaCO_3{\cdot}6H_2O$).[42] However, this compound is very unstable and loses its crystal water even at 5 °C.[44] Note that the reference compound monohydrocalcite itself has never been studied by EXAFS until now, although its crystal structure has been used for EXAFS fits.[21,24,27] It must also be noted that the coordination number given by EXAFS is of limited accuracy due to its strong mathematical correlation with the Debye–Waller factor σ^2 and the amplitude reduction factor S_0^2.[45]

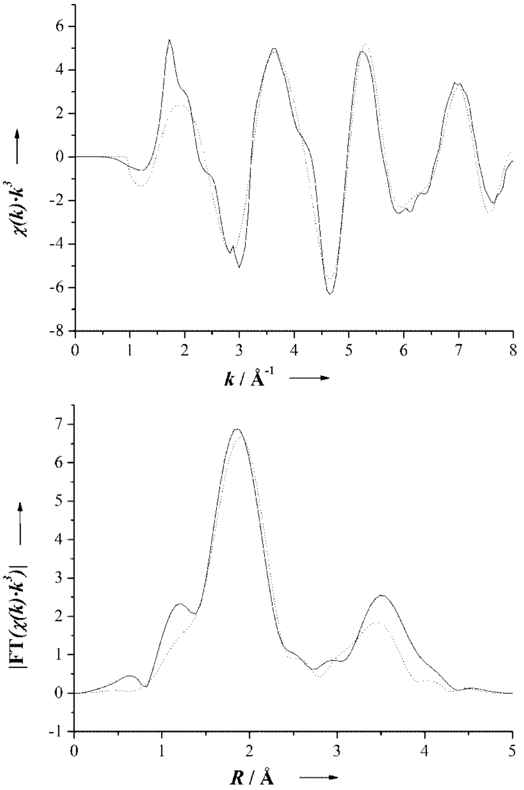

Figure 6. Ca *K*-edge EXAFS spectra of monohydrocalcite. Top: raw EXAFS data $\chi(k)$; bottom: Fourier transform magnitude. Solid line: experimental data; dotted line: fit data.

Crystallographically,[9] the environment of Ca in monohydrocalcite consists of eight oxygen atoms at distances of 2.41–2.49 Å (six from carbonate and two from hydrate water), two carbon atoms at a distance of 2.78–2.90 Å, two carbon atoms at a distance of 3.15–3.33 Å, one oxygen atom at a distance of 3.60 Å, and two calcium atoms at a distance of 3.86 Å. The higher coordination number in the first Ca–O shell (eight vs. six for calcite and vaterite, and nine for aragonite) and the lower coordination number in the outer Ca–Ca shell (two vs. six for calcite, aragonite and vaterite) distinguish monohydrocalcite from the water-free phases. The fact that the outer Ca–Ca shell in ACC is generally weak or not even present points to some similarity of monohydrocalcite to ACC, thus making it a useful structural model. An important difference, however, is the distance of the first Ca–O shell, which is very charac-

Table 1. Results reported in the literature for EXAFS spectra of X-ray-amorphous calcium carbonates (ACC) found naturally, together with crystalline phases. The entries are sorted by ascending distance of the first coordination shell of oxygen. Italicised values were kept constant during the EXAFS fit.

	First shell: Ca–O			Second shell: Ca–C			Third shell: Ca–O			Fourth shell: Ca–Ca		
	R [Å]	N	$\sigma^2 \cdot 10^3$ [Å^2]	R [Å]	N	$\sigma^2 \cdot 10^3$ [Å^2]	R [Å]	N	$\sigma^2 \cdot 10^3$ [Å^2]	R [Å]	N	$\sigma^2 \cdot 10^3$ [Å^2]
ACC in plant cystoliths of *Ficus microcarpa*[24]	2.20 2.41	2.3 3.3	5 0.2	3.50	*2*	40	–[a]			3.79	4	13
Calcite[28]	2.34	*6*	9	3.30	*6*	35	3.64	6	16	4.05	6	9
ACC in carapaces of *Homarus american*[24]	2.23 2.41	1.9 3.9	5 3	3.47	*4*	13	–[a]			3.79	*2*	13
ACC in plant cystoliths of *Ficus retusa*[42]	2.35 2.43 2.44 2.73	2 2 2 2	17 29 34 47	3.20	1.5	31		–[a]			–	
Vaterite[28]	2.37	6	7	3.09	*2O, 2C*	21	–	–	–	4.24	*6*	15
Cuticula of *Porcellio scaber* (calcite + ACC)[43]	2.37	5.1	10	2.99	2.2	48	3.64	1.0	0	4.05	*6*	38
Cuticula of *Armadillidum vulgare* (calcite + ACC)[43]	2.37	6.6	12	3.00	2.4	–23	3.64	0.8	0	4.02	6	35
ACC in spicules of *Pyura Pachyderma tina*[21]	2.37	7.4	9	3.03	1.5	7	3.36	3	0	–[a]		
ACC in sternal deposits of *Porcellio scaber*[28]	2.38	3.8	14	3.00	1.4	7	3.72	0.4	1	(3.89)	*6*	109
ACC prepared in vitro[36]	2.41	5.3	11	–[a]			–[a]			–[a]		
Monohydrocalcite (this work)	2.42	8.2	14	3.04	*4*	22	–[a]			3.83	*2*	5
ACC in larval shells of *Biomphalaria glabrata*[20,29]	2.44	9	31	–[a]			–[a]			3.92	*6*	47
Aragonite[28]	2.47	*9*	33	2.98	*3*	3	3.26	3	10	3.94	*6*	28

[a] Shell was not included in the fit.

teristic and can be determined with high accuracy by EXAFS. This shows that not all ACC phases that have been described have a short range like that of monohydrocalcite.

Scanning electron microscopy on monohydrocalcite showed that it consists of platelets that are aggregated into larger particles (Figure 7), whereas the ACC prepared by Faatz et al.[35] and Günther et al.[36] shows a spherical morphology with sub-micrometer-sized particles, therefore there is no similarity in the morphology of monohydrocalcite and ACC prepared in vitro.

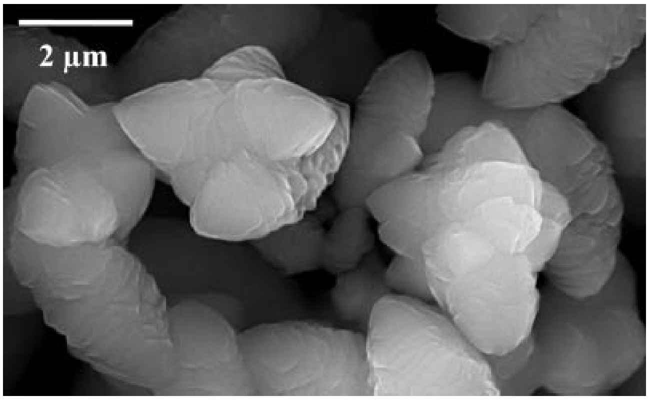

Figure 7. Scanning electron micrograph of synthetic monohydrocalcite.

Conclusions

Monohydrocalcite as a stable hydrated phase of calcium carbonate is a suitable model for biogenic amorphous calcium carbonate (ACC) with regards to its composition (one water molecule per calcium carbonate unit). However, its microstructure, as determined by EXAFS, does not match the structure of all biogenic or synthetic ACC phases. This may be related to a higher degree of order in this crystalline phase, which also leads to a higher dehydration temperature compared to ACC. It may be assumed in the light of new theories on the formation of biominerals[18,19] that nanoscopic particles of hydrated calcium carbonate (resembling monohydrocalcite) assemble into water-containing ACC but do not crystallise (as shown by the fact that biogenic ACC is X-ray-amorphous), which means that the aggregates possess a higher energy than crystalline monohydrocalcite. Such biogenic assemblies always contain some organic material, which apparently prevents this ordering process. Unfortunately, no quantitative data on the content of organic matrix in biogenic ACC is available. The fact that many biogenic ACC phases are probably hydrated (although, to the best of our knowledge, this has only been quantified in one case by thermogravimetry[21] due to the small amount of sample material available) but structurally different[27,36,43] points to either different nanoscopic precursor particles or to a rearrangement during macroscopic assembly and ordering.

Experimental Section

Preparation of Monohydrocalcite: Artificial seawater was prepared according to Kralj and Brecevic[4] by dissolving the following amounts of chemicals in 250 mL of doubly distilled water: 7.30 g of sodium chloride, 1.25 g of magnesium chloride, 0.370 g of calcium chloride dihydrate and 0.006 g of strontium chloride. Potassium carbonate (0.3 g) was then added and the solution was stirred until it had dissolved. The mixture was then placed in an ultrasonic bath for 10–15 s and left in a refrigerator at 6 °C overnight. The precipitate of monohydrocalcite obtained was filtered off and dried in air. Elemental analysis by combustion analysis (C, H) and atomic absorption spectroscopy (Ca, Mg) gave the following results: calcd. C 10.17, H 1.71, Ca 33.94, Mg 0; found C 10.06, H 1.83, Ca 33.67, Mg 0.32.

Analytical Techniques: Combined thermogravimetry/IR spectroscopy (TG-IR) was carried out with a Netzsch STA 209 TG-DTA/DSC instrument connected to a Bruker Vertex 70 IR spectrometer for gas analysis in situ. The sample was heated from 30 to 900 °C at a rate of 5 $°C\,min^{-1}$ under N_2 (50 $mL\,min^{-1}$). Scanning electron microscopy was carried out with a Zeiss DSM 962 instrument on Au-sputtered samples. X-ray powder diffractometry was carried out with a Stadi P instrument from STOE with Cu-K_α radiation. The DSC measurement was carried out with a Netzsch DSC 204 instrument. The sample was heated from 0 to 500 °C at a rate of 5 $°C\,min^{-1}$. Extended X-ray absorption fine structure (EXAFS) spectroscopy was carried out at beamline E4 at the Hamburger Synchrotronstrahlungslabor (HASYLAB) at the Deutsches Elektronen Synchrotron (DESY), Hamburg, Germany. The programs SPLINE/XFIT[46] and FEFF 6.01a[47] were used for quantitative data evaluation. The amplitude reduction factor S_0^2 was fixed to 0.8. Variation parameters were the bond lengths for all shells, the coordination numbers (only for the first shell), the Debye–Waller factors (σ^2) for all shells, and the zero-energy correction (E_o).

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