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On the Structure of Amorphous Calcium Carbonate—A Detailed Study by Solid-State NMR Spectroscopy

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The calcium carbonate phases calcite, aragonite, vaterite, monohydrocalcite (calcium carbonate monohydrate), and ikaite (calcium carbonate hexahydrate) were studied by solid-state NMR spectroscopy (^{1}H and ^{13}C). Further model compounds were sodium hydrogencarbonate, potassium hydrogencarbonate, and calcium hydroxide. With the help of these data, the structure of synthetically prepared additive-free amorphous calcium carbonate (ACC) was analyzed. ACC contains molecular water (as H_2O), a small amount of mobile hydroxide, and no hydrogencarbonate. This supports the concept of ACC as a transient precursor in the formation of calcium carbonate biominerals.

Introduction

Calcium carbonate is the most prominent biomineral in terms of global turnover.[1] Technically, it is also of major importance with respect to the scaling of heaters and pipes. Calcium carbonate occurs in three anhydrous polymorphic forms (calcite, aragonite, and vaterite), where the thermodynamic stability decreases from calcite to vaterite.[2] In addition, two crystallographically defined pseudopolymorphs are known: monohydrocalcite, $CaCO_3 \cdot H_2O$,[3−5] and the hexahydrate form, $CaCO_3 \cdot 6H_2O$, the mineral ikaite.[4,6] Amorphous calcium carbonate (ACC) with variable composition is also well known. In the biological formation of hard tissue, for example, in biomineralization, control over the polymorphic phase is of particular importance and typically under strict biological control.[2] It was shown that amorphous calcium carbonate can act as a precursor to the crystalline phases; that is, it undergoes a structural transformation. Specialized biomolecules are able to induce the formation of amorphous calcium carbonate[7,8] and also to stabilize it as a storage phase[9−12] or as a transient phase.[13−15]

However, not all samples of ACC have the same internal structure, as was demonstrated by X-ray absorption spectroscopy (EXAFS); the way of its formation, that is, the biological system or the chemical method, strongly influences the short-range order around calcium and its water content.[5,16−18] It was shown by EXAFS that in some cases the internal structure of the subsequently forming crystalline calcium carbonate phase was already "imprinted" into the structure of the initial ACC precursor phase.[19−22] In terms of chemical composition, biogenic ACC always seems to

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contain water. A structural relationship to monohydrocalcite,[5] that is, calcium carbonate monohydrate, was suggested on the basis of EXAFS results[16,20] and on the analysis of the water content. Biogenic ACC from the spicules of *Pyura pachydermatina* contains 15.7 wt % water, close to a monohydrate.[20] We have shown recently that the EXAFS spectrum of monohydrocalcite matches some of the known ACC phases, but not all of them.[5]

Solid-state NMR spectroscopy is a very useful method to analyze X-ray amorphous or nanoparticulate compounds where diffraction methods are not applicable for structure elucidation. Takahashi et al. have studied the interface between biomolecules and aragonite in nacre.[23] Jaeger and Coelfen have discovered recently by solid-state NMR spectroscopy (^{13}C and ^{1}H) that there is a thin layer of ACC around the aragonite platelets in nacre.[24,25] For nanocrystalline calcium phosphate, which is the mineral in mammalian bone,[26,27] information on the surface phases of such nanoparticles and their interaction with the surrounding tissue can be obtained.[28,29] In biogenic calcium phosphate samples (like bone), ^{31}P solid-state NMR spectroscopy has provided deep insights,[30–32] and for silica-containing organisms, ^{29}Si solid-state NMR spectroscopy has been successfully employed.[33–35] Cho et al. have measured the hydroxide content in bone mineral (apatite) by ^{1}H solid-state NMR spectroscopy and found considerably less than expected by stoichiometry.[36]

The analysis of amorphous calcium carbonate from biological sources is difficult because it generally occurs together with an organic matrix. In addition, the available amounts are very small and not sufficient for solid-state NMR experiments. There are also a number of procedures to prepare ACC in vitro.[37–39] We have therefore prepared amorphous calcium carbonate *in vitro* and analyzed it by solid-state NMR spectroscopy. No additives were present during the preparation, as only calcium hydroxide and carbon dioxide were used as parent compounds; that is, there are no impurities which disturb the NMR analysis.

Experimental Section

The calcium carbonate phases were prepared in a self-designed crystallization apparatus (see ref 40 for details on the preparation). Calcite was prepared by rapidly mixing 2 M $CaCl_2$ (pH 2.7) and 0.05 M Na_2CO_3 (pH 11.4) at 40 °C, followed by stirring for 6 h. Aragonite was prepared by rapidly mixing 0.1 M $CaCl_2$ (pH 4) and 0.1 M Na_2CO_3 (pH 11.4) at 80 °C, followed by immediate filtration. Vaterite was prepared by rapidly mixing 2 M $CaCl_2$ (pH 2.7) and 0.05 M Na_2CO_3 (pH 11.4) at 1 °C, followed by immediate filtration. Calcite, aragonite, and vaterite were all dried at 70 °C in the air. Monohydrocalcite was prepared according to ref 5 by precipitation from artificial seawater (containing NaCl, $MgCl_2$, $CaCl_2$, $SrCl_2$, and K_2CO_3) and dried at room temperature in the air. ACC was prepared according to ref 39 from $Ca(OH)_2$ and gaseous CO_2. It was dried at 70 °C in the air. In an earlier study by synchrotron X-ray powder diffraction, we have demonstrated the absence of any diffraction peaks, that is, its completely amorphous nature, which was confirmed here by a diffraction experiment on a laboratory diffractometer.[39] Ikaite was prepared in the same way as ACC, but the precipitate was not dried in a vacuum after washing with acetone, but immediately put into a freezer at −20 °C.

All chemicals used were of p.a. quality. Ultrapure water was prepared with an ELGA Purelab ultra instrument and used for all syntheses. The purities of $NaHCO_3$ (Fluka), $KHCO_3$ (Riedel-de Haen), and $Ca(OH)_2$ (Merck) were controlled beforehand by X-ray powder diffraction.

Scanning electron microscopy (SEM) was performed with a FEI Quanta 400 ESEM instrument with gold−palladium alloy sputtering. Thermogravimetry−differential thermal analysis (TG-DTA) was carried out with a Netzsch STA 209 TG-DTA/DSC instrument. The samples were heated at a rate of 1 K min^{-1} under a dynamic N_2 atmosphere (50 mL min^{-1}) from 25 to 1000 °C to obtain their composition (water and carbonate contents). X-ray powder diffraction was carried out with a Siemens D500 diffractometer operating with Cu Kα radiation (λ = 1.5406 Å) at 40 kV and 20 mA in Bragg-Brentano mode with a step size of 0.02° 2Θ and a counting time of 1 s per step. Calcium, magnesium, and sodium were determined by atomic absorption spectroscopy (Thermo Electron Corporation, M-Series AA spectrometer). Infrared spectroscopy (FTIR) was performed with a Bruker-Vortex 70 instrument in KBr pellets. Carbon and hydrogen were determined by standard combustion analysis with an EA 1110 (CE Instruments) instrument. All elemental analyses were performed twice.

Solid-state NMR spectroscopy was performed on a Bruker ASX 400 spectrometer with a 400.132 MHz resonance frequency for protons and 100.623 MHz for carbon nuclei. For all solid-state spectra, magic angle spinning (MAS) was applied at frequencies of 4000 Hz in a 7 mm rotor for carbon and 15 000 Hz in a 4 mm rotor for proton measurements. For ^{13}C MAS NMR spectra, the

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Table 1. Structure and Composition of All Investigated Calcium Carbonate Phases[a]

phase	calcite $CaCO_3$	aragonite $CaCO_3$	vaterite $CaCO_3$	monohydrocalcite (MHC) $CaCO_3 \cdot H_2O$	ikaite $CaCO_3 \cdot 6\ H_2O$	amorphous calcium carbonate (ACC)
typical particle size and morphology (SEM)	1−5 μm (rhombohedral)	needles of 0.2−0.4 μm thickness and 2−4 μm length	100 nm (spherical)	2−4 μm (aggregates of platelets)	platelets of 2−4 μm thickness and 15−18 μm length	80−120 nm (spherical)
calcium/wt % (AAS)	40.0(2)	40.0(2)	35.4(5)	33.7(2)		35.2(2)
magnesium/wt % (AAS)				0.32(8)		
sodium/wt % (AAS)	0.040(2)	0.75(3)	4.1(3)			
carbon/wt % (combustion analysis)	11.89(2)	11.91(4)	11.78(2)	10.06(1)		10.81(2)
hydrogen/wt % (combustion analysis)	0.17(1)	0.13(1)	0.27(1)	1.83(2)		1.02(1)
water content/wt % (TGA)	0.0	0.0	0.8	14.9		7.5
carbon dioxide release/ wt % (TGA)	43.8	42.0	38.9	36.4		38.9
computed formula, based on elemental analysis (Ca, Mg, Na, C, H)	$Ca(CO_3)_{0.991} \cdot 0.001\ Na_2CO_3$	$Ca(CO_3)_{0.956} \cdot 0.016\ Na_2CO_3$	$Ca(CO_3)_{1.000} \cdot 0.102\ Na_2CO_3 \cdot 0.050\ H_2O$	$CaMg_{0.016}(CO_3)_{0.985} \cdot 0.984\ H_2O$		$Ca(CO_3)_{1.005} \cdot 0.473\ H_2O$

[a] A detailed elemental analysis showed that small amounts of incorporated ions or X-ray amorphous phases are present, despite the crystallographic phase purity of the samples. For ikaite, no proper elemental or thermogravimetric analysis was possible due to rapid thermal degradation (a loss of water above 0 °C). In all cases, less than the expected six water molecules were found. All standard deviations are given in parentheses.

direct excitation of ^{13}C nuclei was induced by a single 30° pulse of 5.75 μs duration. Generally, the fully decoupled free induction decay was accumulated over 172 to 640 scans and Fourier-transformed in order to obtain the line spectrum. A waiting period of 180 s was used between the experiments to allow for full spin-lattice relaxation. For ^{13}C CPMAS (cross-polarization MAS) NMR spectra, $^1H-^{13}C$ cross-polarization was induced by full polarization of the protons and by adjusting the Hartmann−Hahn condition over a contact period of 4 ms. In this case, the fully decoupled free induction decay was accumulated typically over 8800 scans. For ^1H MAS NMR spectra, direct excitation of the protons was induced by a single 90° pulse. Typically, the free induction decay was accumulated over 128 to 256 scans and Fourier-transformed in order to obtain the line spectrum. A waiting period of 5 s was used between the experiments to allow for full spin-lattice relaxation. A static control experiment was carried out with ACC to elucidate the nature of the narrow peak at 2.32 ppm; that is, a stimulated echo sequence together with a pair of pulsed field gradients was applied in order to study the diffusion properties of the corresponding component. All experiments were carried out at ambient temperature, except for the case of ikaite, which was studied at −20 °C. Special care was taken to avoid warming and the resulting decomposition of ikaite during all experimental procedures. Due to the limited amount of available monohydrocalcite, the NMR rotor was partially filled with polytetrafluoroethylene (PTFE) as a spacer. PTFE gives a signal in ^{13}C MAS NMR spectroscopy but not in ^{13}C CPMAS NMR or ^1H MAS NMR spectroscopy.

Results and Discussion

All polymorphs and pseudopolymorphs of calcium carbonate were prepared and thoroughly characterized with respect to their chemical and crystallographic purity (Table 1). The particle size and morphology are also given here, so that effects of the particle surface can be better judged. Note that the surface layer of small particles can be quite different from the bulk phase, as has been shown previously for nanohydroxyapatite.[31] Thorough elemental analyses revealed that the samples of calcite, aragonite, and vaterite contained traces of sodium from the preparation, either as ionic substitution into the calcium carbonate lattice or as amorphous sodium carbonate. The sample of ACC contained about 0.5 mol of water per calcium carbonate unit, that is, less than monohydrocalcite. As it was dried at 70 °C and as it loses its water in TGA between 100 and 260 °C, we can safely assume that this water content is structurally incorporated and not simply adsorbed.[39] Upon contact with liquid water or complete removal of the incorporated water, this ACC crystallized to calcite; therefore, we assume a "calcitic" internal structure.[39]

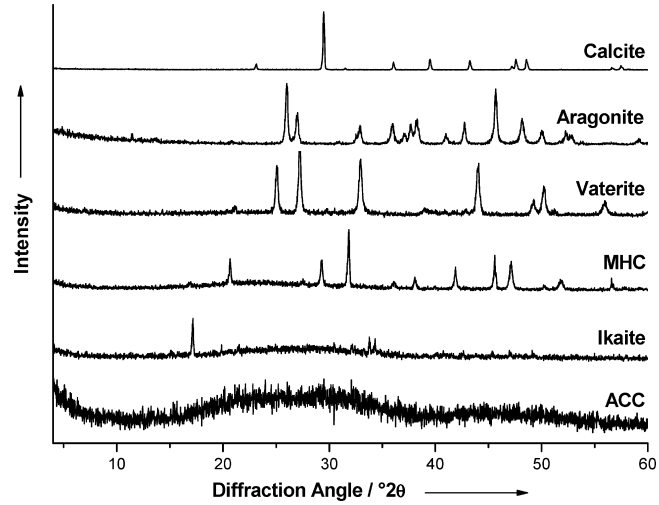

Figure 1. X-ray powder diffraction patterns of all studied calcium carbonate phases. Only the expected peaks with respect to the crystal structures given in the literature and the JCPDS database were found. ACC is completely X-ray amorphous, in accordance with earlier results.[39] Note that the intensity axis is scaled differently for each phase; that is, the relative height of each diffractogram corresponds to its strongest diffraction peak.

All samples were crystallographically pure; that is, no crystalline foreign phases were found (Figure 1). This was confirmed by Rietveld refinement, as reported earlier.[40] With the exception of ACC, they all had a high crystallinity, as indicated by narrow diffraction peaks. We conclude that the ionic impurities (Na and Mg) are either present as ionic substitutions in the calcium carbonate lattice or possibly in an X-ray amorphous state of their carbonates. As these

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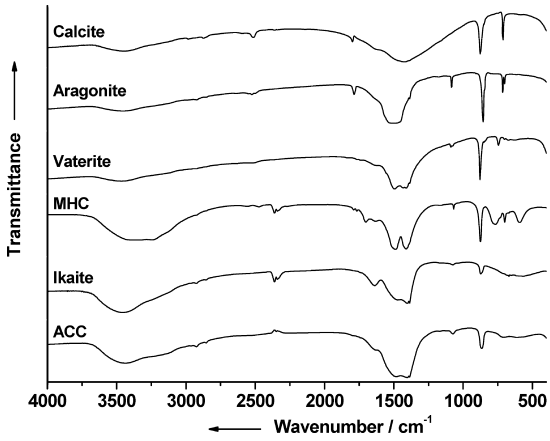

Figure 2. Infrared spectra of all studied calcium carbonate phases. All bands correspond to the tabulated values[41] (see also Table 2).

impurities are minor and not crystallographically detectable, it can be safely assumed that they did not influence the NMR results. We wish to note that such a rigorous phase analysis is typically not performed when such samples are prepared and analyzed (very often, only X-ray powder diffraction data are reported). The infrared spectra also corresponded to the theoretical values (Figure 2 and Table 2).[41]

The internal structure of all phases was studied by solid-state NMR spectroscopy. The NMR data are summarized in Table 3. The ^{13}C NMR spectra are shown in Figures 3 and 4. All samples showed only carbonate or hydrogencarbonate peaks in the range of 156−174 ppm. The crystalline, water-free phases calcite and aragonite showed very narrow NMR peaks, whereas the water-containing phases all showed broad peaks. The line width of the ACC peak was especially large, as expected due to the disordered structure. There is a clear distinction between the regions of carbonate peaks (about 166 to 174 ppm) and hydrogencarbonate peaks (below 166 ppm). We conclude that the majority of the carbon atoms in ACC is present as carbonate and not as hydrogencarbonate, with some similarity of the chemical environment to the highly hydrated phase ikaite.

In the ^{13}C CPMAS spectra, only carbon atoms with protons in their vicinity are visible. Consequently, no signal is observable for the water-free phases calcite and aragonite, whereas phases that contain either hydrogencarbonate or water show distinct peaks (Figures 5 and 6). Again, the peak in ACC is clearly due to incorporated water and not to hydrogencarbonate, and the chemical shift is close to that in the water-rich phase ikaite. Vaterite contained traces of water, which show up in the CPMAS spectrum.

The results correspond well to the literature. Papenguth et al. have studied a number of calcium carbonate phases of biological and geological origin by ^{13}C MAS NMR spectroscopy. For pure aragonite, they found a single peak at 169.9 ppm (fwhm 1.2 ppm); for pure calcite, they found a single peak in the range of 167.4−167.9 ppm (fwhm 1−1.1 ppm); for vaterite containing 9 % calcite, they found a single peak at 168.7 ppm (fwhm 1.9 ppm); for crystalline magnesian calcite, they found a single peak in the range of 167.2−167.5 ppm (fwhm 1.7−2.4 ppm), and for a mixture of amorphous and crystalline magnesian calcite, they found a broad peak at 166.3 ppm (fwhm 7.7 ppm).[42] Feng et al. have found traces of hydrogencarbonate in crystalline calcite by enrichment with ^{13}C. They concluded that, in ^{13}C NMR spectroscopy, carbonate cannot be distinguished from hydrogencarbonate in the case of calcite (a single peak at 168.7 ppm with fwhm < 0.1 ppm).[43] However, this may be due to the very small amount of hydrogencarbonate in calcite (content about 70 $\pm$ 20 ppm) where the crystallographic environment of hydrogencarbonate is practically the same as in pure calcite. Nassif et al. have shown for biogenic aragonite from nacre of *Haliotis laevigata* that the ^{13}C aragonite resonance occurs around 171 ppm for the crystalline phase and, by about 0.5 ppm, shifted to lower ppm values for amorphous ACC at the surface of the aragonite tablets. They performed a ^{13}C CPMAS NMR experiment with magnetization transfer from the surrounding organic phase.[25]

Ikaite showed a slow decomposition during the NMR experiment, although it was carried out at −20 °C. After the experiment, it had partially converted to calcite, as confirmed by X-ray powder diffraction. Due to the fact that we still observed a major CPMAS signal in contrast to calcite, we tentatively assume that the ^{13}C NMR spectra still correspond to pure ikaite and that the conversion to calcite occurred during emptying of the NMR rotor, subsequent grinding, and X-ray diffraction analysis, all being performed at room temperature. In any case, the ^{13}C chemical shift is very close to that of calcite. All other phases (including ACC) were studied by X-ray diffraction after the NMR experiments and showed no change in either crystallinity or phase composition.

By ^{1}H NMR, the nature of the incorporated hydrogen can be elucidated. The ^{1}H signal for hydrogencarbonate occurs above 13 ppm, and that for incorporated molecular water occurs between 5 and 5.6 ppm. Hydroxide occurs between 1.5 and 2.4 ppm. Gaffey found by ^{1}H CRAMPS-NMR spectroscopy that incorporated water in biogenic magnesium-containing calcite (from echinoderm skeletons) gives a peak around 5 ppm. Hydroxide ions occurred in the region of 0−1 ppm for the mineral brucite, $Mg(OH)_2$.[44] By ^{1}H NMR spectroscopy on nacre, Nassif et al. found mobile water at 5.2 ppm and hydrogencarbonate at 14.4 ppm.[25] By ^{1}H NMR on ^{13}C-enriched calcite, Feng et al. found traces of hydrogencarbonate (content about 70 $\pm$ 20 ppm) at 7.4 ppm, water at 4.8−6 ppm, and hydroxide at 0−2 ppm. For $NaHCO_3$, they found the hydrogencarbonate peaks at 14.1 ppm and for $Na_3(HCO_3)(CO_3)(H_2O)_2$ at 18.6 ppm.[43] The ^{1}H chemical shift of hydrogencarbonate is strongly dependent on the chemical environment of the protons, that is, mainly on the strength of hydrogen bonds and on the O−H···O bond length.[43] However, a signal at 5 ppm as observed here for ACC is clearly out of the range of the corresponding

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Table 2. Assignment of the IR Bands of the Investigated Calcium Carbonate Phases[41]

IR band	calcite	aragonite	vaterite	MHC	ikaite	ACC
ν_3 (asymmetric C−O-stretching)	1421	1506	1495, 1433, 1412	1489, 1412	1474, 1402, 1385	1483, 1404, 1385
ν_1 (symmetric C−O-stretching)		1084	1088	1068	1074	1074
ν_2 (CO_3^{2-}-bending)	876	856	878	874	872	866
ν_4 (O−C−O-bending)	712	714	744	766, 700	714, 669	714, 700

Table 3. Results of the NMR Data of All Phases[a]

phase	calcite	aragonite	vaterite	monohydrocalcite	ikaite	ACC	$NaHCO_3$	$KHCO_3$	$Ca(OH)_2$
^{13}C-MAS/chemical shift	168.21 (CO_3^{2-})	170.49 (CO_3^{2-})	170.12, 169.07 (CO_3^{2-})	171.10 (CO_3^{2-})	167.98 (CO_3^{2-})	167.98 (CO_3^{2-})	164.47 (HCO_3^-)	161.94 (HCO_3^-)	
^{13}C-MAS/line width	0.25	0.4	0.7, 0.6	2.1	0.9	3.5	2.2	5.7	
^{13}C-CPMAS/chemical shift			169.70 (CO_3^{2-})	171.19 (CO_3^{2-})	167.83 (CO_3^{2-})	167.99 (CO_3^{2-})	164.32 (HCO_3^-)	161.15 (HCO_3^-)	
^{13}C-CPMAS/line width			2.1	0.7	1.0	3.0	2.1	1.1	
^{1}H-MAS/chemical shift			17.92, 15.75 (weak; HCO_3^-)				14.16 (HCO_3^-)	13.56 (HCO_3^-)	
			5.56 (H_2O)	5.43(H_2O)		5.01 (H_2O)		5.13 (H_2O)	
			2.34, 1.56 (weak; OH^-)	1.55 (weak; OH^-)		2.32 (sharp; OH^-),1.53 (weak; OH^-)			1.57 (OH^-)
^{1}H-MAS / line width (given for all listed peaks from high to low ppm values)			1.5,1.5,1.8, n/a, 0.2	1.6, 0.1		1.8, 0.05, 0.1	1.2	1.4, 0.1	7.8

[a] All values are given in ppm. The line width is given in ppm as the full width at half maximum (fwhm).

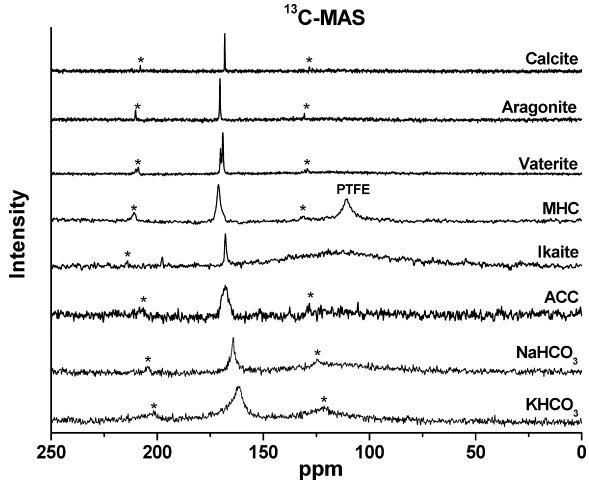

Figure 3. ^{13}C MAS NMR spectra of all investigated phases and the two reference compounds sodium hydrogencarbonate and potassium hydrogencarbonate. The peak at 110.48 ppm in monohydrocalcite is due to polytetrafluoroethylene (PTFE), which was used as filling material due to the small amount of available sample. "*" indicates spinning side bands. Note that the intensity axis is scaled differently for each phase; that is, the relative height of each spectrum corresponds to the strongest peak.

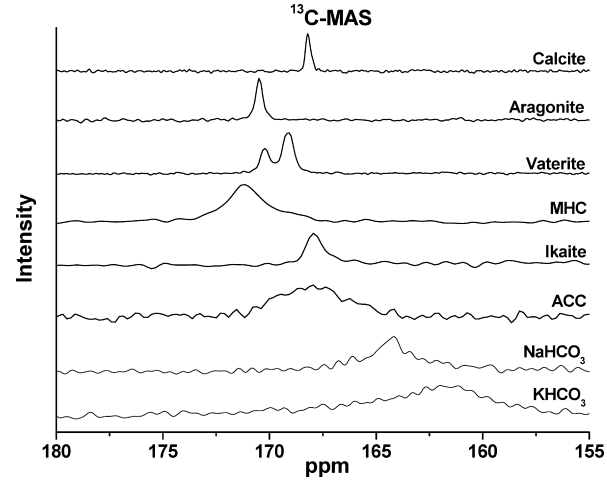

Figure 4. Enlargement of the carbonate region of the ^{13}C MAS NMR spectra from Figure 3.

correlation. Therefore, we ascribe this signal to incorporated molecular water, that is, H_2O. The peaks between 1.5 and 2.5 are due to traces of a mobile water fraction containing hydroxide ions. This is supported by the reference compound calcium hydroxide (1.57 ppm) and was also confirmed by a static experiment on ACC. Under the influence of a pair of pulsed field gradients in combination with a stimulated echo sequence, the narrow peak showed a rapid decay with increasing gradient strength. Even with a waiting period of only 15 ms, the signal intensity was reduced to the noise level at a gradient strength of only 100 G cm^{-1}. From this observation, we conclude that the corresponding component must be of a very small molecular mass. In addition, it must occur in a dissolved state allowing for a relatively free diffusion process within the sample.

Vaterite, monohydrocalcite, and ACC all contain molecular water as the main hydrogen component and only traces of hydroxide. In the vaterite sample, a small amount of hydrogencarbonate is also present, possibly due to the incorporated sodium.

Conclusions

The polymorphs and pseudopolymorphs of calcium carbonate can be distinguished by their NMR spectra. They all contain carbonate ions, which result in a distinct chemical shift in ^{13}C NMR spectroscopy, depending on their crystallographic environment. ^{13}C CPMAS cross-polarization allows a distinguishing of water-containing phases from water-free phases. In the case of the hydrate phase monohydrocalcite, molecular water is detected by ^{1}H NMR spectroscopy. The X-ray amorphous, structurally largely unknown phase ACC

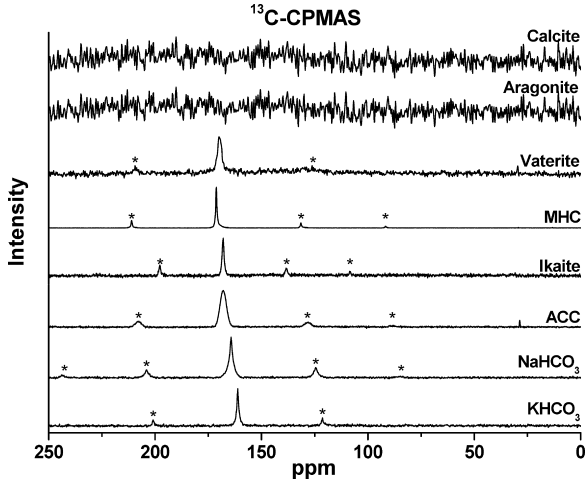

Figure 5. ^{13}C CPMAS NMR spectra of all investigated phases. Due to the cross polarization, carbon atoms in the vicinity of hydrogen atoms show an amplified intensity, and the carbonate signals of calcite and aragonite have vanished. "*" indicates spinning side bands. Note that the intensity axis is scaled differently for each phase; that is, the relative height of each spectrum corresponds to the strongest peak.

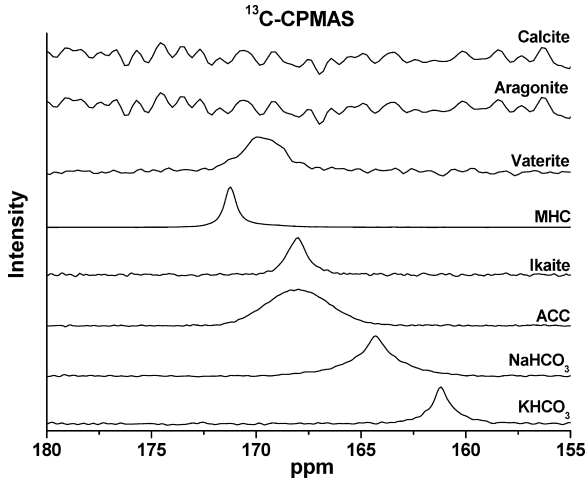

Figure 6. Enlargement of the carbonate region of the ^{13}C CPMAS NMR spectra from Figure 5.

contained about 0.5 mol of structural water per calcium carbonate unit. This is mainly present as molecular water with variable mobility and as traces of mobile ("dissolved") hydroxide anions, but not as hydrogencarbonate. This supports the concept of a water-containing precursor phase, which can be biologically converted into the crystalline calcium carbonate phases by structural reorganization and a simultaneous loss of incorporated water. The conversion of

(44) Gaffey, S. J. *Am. Mineral.* **1995**, *80*, 947–959.

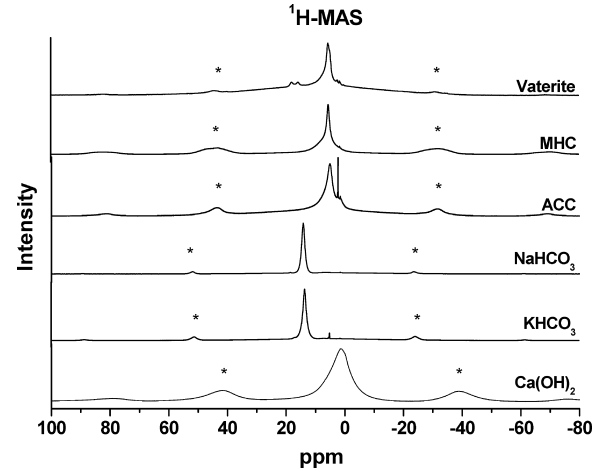

Figure 7. ^{1}H MAS NMR spectra of all phases. "*" indicates spinning side bands. Note that the intensity axis is scaled differently for each phase; that is, the relative height of each spectrum corresponds to the strongest peak.

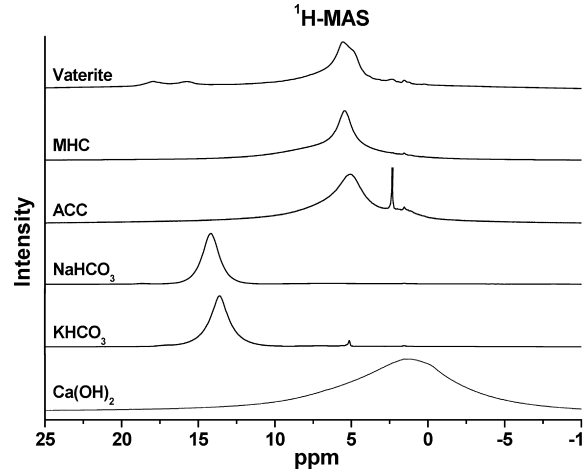

Figure 8. Enlargement of the ^{1}H MAS NMR spectra from Figure 7. The narrow peak observed for ACC at 2.32 ppm results from a small mobile water fraction which contains dissolved hydroxide ions.

hydrogencarbonate to carbonate would require a much larger activation energy that is difficult to overcome during biomineralization processes that occur near ambient temperatures.

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