Effect of Serine and Arginine on the Phase Transition from Amorphous CaCO_3 and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ to Calcite Film

Seung-Woo Lee^{1,2}, Yong-Il Kim³, Kyubock Lee⁴, Jun-Hwan Bang¹,
Chi-Wan Jun¹ and Young-Nam Jang^{1,2,*}

¹*CO₂ Sequestration Research Department, Korea Institute of Geoscience and Mineral Resources, Gwahangno 92, Yuseonggu, Daejeon, 305-350, Korea*

²*Resource Recycling, University of Science and Technology, Daejeon, Korea*

³*Korea Research Institute of Standards and Science, 1, Doryoung-dong, Yuseonggu, Daejeon, 305-340, Korea*

⁴*Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany*

Calcium carbonate, ubiquitous throughout nature, is one of the most biologically significant minerals. It is known that the organic matrix of biological materials controls the phase transition of CaCO_3 , but much remains undiscovered regarding its pathway from an amorphous to crystalline solid. In this study, examination of the initial formation of CaCO_3 films has been proposed as a new methodology to identify the phase transition of CaCO_3 . We have identified a significant role for both serine and arginine in the synthesis of CaCO_3 and have found them to be important for the stabilization of amorphous calcium carbonate and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$. X-ray diffraction, scanning electron microscopy, and transmission electron microscopy have been used for the identification of crystalline phase and surface structure. This study presents information useful for understanding the phase transition of CaCO_3 and the function of organic molecules in the formation of biological materials.

[doi:10.2320/matertrans.M2012164]

(Received May 2, 2012; Accepted July 13, 2012; Published September 25, 2012)

Keywords: amorphous CaCO_3 (ACC), $\text{CaCO}_3 \cdot \text{H}_2\text{O}$, $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$, calcite, phase transition, serine, arginine

1. Introduction

Biological materials including bone, mollusk shells, and others are synthesized by many different organisms.^{1,2)} They have received significant interest in the fields of chemistry and materials science due to their higher material properties. Mineral materials, including calcium- and silicon-based biomaterials, are commonly produced by a wide range of phyla, including archaea, bacteria, fungi, lower and higher plants, and the Chordata. Calcium carbonate (CaCO_3), which forms the exoskeleton of mollusk shells and sponges, has been a subject of study due to its wide availability and excellent material characteristics.^{3,4)} In particular, the shell formation of bivalves has been used as a means to understand the mechanisms of biomineralization.

In natural systems, CaCO_3 generally exists in six forms: three different polymorphs (vaterite, aragonite and calcite), two different hydrated phases (monohydrocalcite, $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ and calcium carbonate hexahydrate, $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$), and amorphous calcium carbonate (ACC).¹⁾ Among these forms, the formation of stable amorphous calcium carbonate is fascinating; its transition to the crystalline phase is not only thermodynamically favored but also kinetically fast.⁵⁾ The occurrence of ACC in nature, such as in bivalve shells or cellular systems, is uncommon. However, the initial biomineralization process of bivalve shell formation indicates the presence of ACC. In bivalve shells, the CaCO_3 crystalline phase starts as ACC, changes to aragonite at the planktonic larval period, and is followed by the transition to calcite after attachment to a substrate.⁶⁾ Thus far, key factors related to the production and retention of ACC have rarely been examined because the biomineralization process has been conducted in closed systems. In these systems, mass transfer is restricted

by an organic membrane or cell, and synthesized biological crystals exist on the micro scale. Therefore, the crystalline phase must be analyzed by X-ray diffraction or other spectrometric techniques.^{7,8)} This study presents methodology that is able to identify the role and effect of amino acids on the synthesis and retention of both ACC and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ in the aqueous state. Using CaCO_3 thin films to understand the mechanism of CaCO_3 phase transitions in nature, we have studied the stabilizing role of amino acids (serine and arginine), a key feature in the transformation pathway of CaCO_3 .

2. Experimental Methods

The method for CaCO_3 film synthesis has been previously described.⁹⁾ CaCO_3 films were obtained under atmospheric conditions (room temperature and 1 atm.). The concentration of amino acids was used to control the ACC (Amorphous Calcium Carbonate) retention period along with the characteristics and morphology of the CaCO_3 thin films. Distilled water (DW), in the absence or presence of amino acids (25 mmole), was mixed with $\text{Ca}(\text{OH})_2$ and stirred at 400 rpm for 1 min. The solution was placed in a 100 ml beaker. The synthesized CaCO_3 films were analyzed after 10 min, 30 min, 1 h, 2 h and 24 h. The synthesized CaCO_3 films were analyzed by X-ray diffraction (DMAX 2200 PV, RIGAKU), field emission scanning electron microscopy (FEI, Nova230 FE-SEM), and transmission electron microscopy (FEI, Tecnai G2 F30 FE-TEM). In the XRD analysis, a Si-low background sample holder was used to decrease the effect of the glass holder. Detailed information on the low-background sample holder is given in the supporting materials (Fig. A2). Appendix contains the crystal patterns of $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$, the Si-low background sample holder information, and the synthetic steps for the CaCO_3 films.

*Corresponding author, E-mail: crystal@kigam.re.kr

Fig. 1 (a) XRD patterns of CaCO_3 thin films synthesized from $\text{Ca}(\text{OH})_2$ and DW (i: 10 min, ii: 30 min, iii: 1 h, iv: 2 h, v: 24 h). The vertical axis represents intensity (cps, counts per second). The star (*) indicates the main peaks of $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$, and the C denotes the characteristic peaks of calcite. ACC is broad peak ranging from approximately 20 to 40°. In the absence of amino acid, the characteristics of ACC were not present. (b) XRD patterns of CaCO_3 thin films synthesized from $\text{Ca}(\text{OH})_2$, serine (25 mM) and DW (i: 10 min, ii: 30 min, iii: 1 h, iv: 2 h, v: 24 h). The vertical axis represents intensity (counts per second). The star (*) indicates the main peaks of $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$, and the C denotes the characteristic peaks of calcite. ACC is a broad peak ranging from approximately 20 to 40°. In the presence of serine, the characteristics of ACC were clearly present compared to the absence of amino acid. (c) XRD patterns of CaCO_3 thin films synthesized from $\text{Ca}(\text{OH})_2$, arginine (25 mM) and DW (i: 10 min, ii: 30 min, iii: 1 h, iv: 2 h, v: 24 h). The vertical axis represents intensity (counts per second). The star (*) indicates the main peaks of $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$, and the C denotes the characteristic peaks of calcite. ACC is a broad peak ranging from approximately 20 to 40°. In the presence of serine (Fig. 1(b)), the characteristics of ACC were clearly present compared to the absence of amino acid.

3. Results and Discussion

To identify the phase transition of CaCO_3 , films synthesized at the interface between DW and air under atmospheric conditions (room temperature and atmospheric pressure) were observed. Figure 1 shows the difference between CaCO_3 crystalline phases with 25 mM serine, 25 mM arginine (Figs. 1(b) and 1(c), respectively), or without additive (Fig. 1(a)). The star (*) indicates the main peaks of $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$, and the C denotes the characteristic peaks of calcite. ACC is a broad peak ranging from approximately 20 to 40°. As shown in Fig. 1, the XRD result in the absence of amino acid (Fig. 1(a)) demonstrates the relatively short residence time of both ACC and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ (dissipation within 30 min after mixing) compared to that observed in the presence of amino acid (Figs. 1(b) and 1(c)). In the case of amino acid addition,

$\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ was identified up to 2 h (25 mM serine) or 1 h (25 mM arginine) post mixing. The crystalline information for $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ was obtained from previous research¹⁰⁾ (Appendix Fig. A1). To eliminate the influence of the amorphous phase in the glass sample holder (also ranging from 20 to 40 degree), a Si-Low background sample holder was used (Appendix Fig. A2).

The retention time of ACC (the broad peak ranging from 20 to 40 degree) was 2 h with the addition of amino acid (Fig. 1). In the CaCO_3 crystallization system of CaCl_2 and NaCO_3 , the complete transition of ACC to calcite has been reported to occur in approximately 4–16 h.¹¹⁾ The transition of ACC to calcite found in natural environments (e.g., mollusk shell formation⁶⁾ or sea urchin larval spicules¹²⁾) shows an increase in the proportion of calcite within an individual species over several days. According to previous research, the intensity of calcite in either the absence or

presence of amino acid increased according to the increase in time after mixing $\text{Ca}(\text{OH})_2$ and DW. The difference in the retention time of the CaCO_3 crystalline phase, ACC and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ was determined by the type of amino acid. To understand these results, correlation of the structural properties between the amino acids (serine and arginine) and the CaCO_3 phase (ACC and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$) ought to be considered. However, a fundamental obstacle faced by structural chemistry has been the lack of a structural model for amorphous CaCO_3 .

Based on the peak ratio of calcite (the final product of the CaCO_3 film), the preferred orientation was identified and is listed according to the amino acid present (Table 1). As shown in Table 1, serine did not develop any specific crystal face compared to the absence of amino acids or the presence of arginine. Arginine developed several crystal faces, including (006) and (018). In particular, the (006) face is equivalent to the (001) face, which is reported to have favorable protein interactions.^{13,14} The experimental results (Fig. 1 and Table 1) showed that the addition of arginine increases the preferred orientation of the (001) face in CaCO_3 films with a calcite crystalline phase. The difference in the preferred orientation has a notable effect on the morphology of CaCO_3 films (Appendix Fig. A3). Moreover, the peak ratio of calcite in the absence of amino acids was very

different from the hexagonal type (PDF 05-0586), which could be due to the morphology differences between burr-like and cubic crystals.

According to the TEM analysis of CaCO_3 synthesized in the absence of amino acids with a 10 min reaction time (Fig. 2), sphere-like particles approximately 1 μm in size were identified as ACC (Fig. 2(b)). The TEM results are consistent with the XRD pattern of CaCO_3 (Fig. 1(a)).

To understand the nature of the starting material for CaCO_3 films, morphological differences were analyzed according to reaction time, ranging from 10 to 30 min (Fig. 3). As shown in Figs. 3(a) and 3(b), the growth and aggregation of particles increased with increasing reaction time (from 10 to 30 min). The shape of the starting material for a film is a hemispherical structure, not a spherical structure (Fig. 3(c)). This result indicates that the source of calcium ions exists only in DW and that the key source of carbonate ions is aqueous CO_2 .

Crystal growth occurs through the aggregation of hemispherical particles. The morphology of calcite thin films depends on the presence of organic molecules. Different shapes, with an aggregated burr- or flower-like structure, were identified depending on the amino acid present (Fig. 4). It is interesting to note that the morphology in the presence of arginine showed a flower-like structure and the absence of amino acids showed a burr-like structure. This difference in morphology may be related to the different degree of preferred orientation in the final crystal structure. In the absence of amino acids, crystal face (202) forms, compared to the presence of amino acids and calcite (Table 1). The correlation between crystal face (202) and burr-like structure could be hypothesized from the degree of preferred orientation. Interestingly, rod-type crystals from urchin teeth showed a developed set of symmetry-related $\{110\}$ planes.¹⁵ The $\{110\}$ plane, including crystal face (202), could be an excellent candidate to develop into stable faces in the burr-like structure of calcite. In the presence of amino acids, crystal faces (006) and (018) developed. As previously mentioned, crystal face (001) is a typical crystal face related to shell formation, composed of calcium carbonate. Nucleation may be associated with amino acids (serine or arginine). In the case of arginine, nucleation may be followed by CaCO_3 growth that develops the more stable (001) and (018) faces in burr-like structures.

Table 1 Degree of preferred orientation. The main peak (104) of calcite was set as a standard for calculation. The degree was calculated to $(\text{hkl})^*100/(104)$. The highest value is indicated in bold.

hkl	None	Serine	Arginine	Calcite*
012	12.3	9.3	5.2	12
104	100	100	100	100
006	<u>3.2</u>	<u>1.5</u>	11.2	<u>3</u>
110	11.5	7.7	2.9	14
113	19.8	15.3	8.6	18
202	23.6	<u>11.6</u>	<u>13.0</u>	<u>18</u>
018	<u>24.7</u>	<u>27.2</u>	47.2	<u>17</u>
116	15.8	16.8	19.0	17
211	2.4	1.8	5.6	4
122	5.9	5.6	2.6	8

*PDF (05-0586)

Fig. 2 TEM images of the starting material for a CaCO_3 film. The material has a spherical structure that is approximately 1 μm in size (a). The diffraction pattern is characteristic of ACC (b).

Fig. 3 SEM images of CaCO_3 . The analysis was conducted according to reaction time after mixing $\text{Ca}(\text{OH})_2$ and DW (a and c: 10 min, b: 30 min) (scale bar: 2 μm (a), 5 μm (b) and (c)). As shown in (c), the CaCO_3 after 10 min contains hemispherical structures.

Fig. 4 Scanning electron microscope images showing developed crystals. (a) is SEM image in the absence of amino acid, (b) and (c) are SEM images of serine (25 mM) and arginine (25 mM), respectively (scale bar: 10 μm (a), 5 μm (b), 5 μm (c)).

ACC is thermodynamically more unstable than calcite but it is a widely found in nature.^{6–8)} Analyses of biological calcium carbonate have identified the presence of phosphate anions and magnesium cations associated with ACC and poorly ordered calcite.^{16–19)}

In general, bivalves use calcium and carbonate ions from seawater for their shell formation. The phase transition of CaCO_3 flows from ACC to aragonite at the stage before attachment and then to calcite at the attachment stage.⁶⁾ It has been reported that various macromolecules (e.g., nacrein, lustrin A, and aspein) can play a key role in the bivalve CaCO_3 phase transition.²⁰⁾

→ Amorphous CaCO_3 → CaCO_3 (aragonite)

→ CaCO_3 (calcite) (Bivalve shell formation)

In contrast, the phase transition of the CaCO_3 films in this study was as follows:

→ Amorphous CaCO_3 & $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$

→ CaCO_3 (calcite) (CaCO_3 film formation).

Initially, ACC and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ were produced and the phase transition to calcite was identified. The presence of serine produced a longer retention time of $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$

than of arginine (Figs. 1(b) and 1(c)). Serine and arginine are thought to prevent crystal nucleation and growth by interfering with the formation of an ordered calcium carbonate crystalline structure. Macromolecules, secreted from epithelial cells, have been reported to inhibit CaCO_3 crystallization, thereby maintaining the ACC state.²¹⁾ Organic molecules shown to promote stable ACC may bind to a specific part of ACC that controls crystallization growth. Detailed investigations of these transformations require the interpretation of ACC and the crystallographic data for amino acids. Evaluating the crystal build of the amorphous phase will be a bottleneck for understanding phase transitions.

Previous research on sea urchins has indicated that transient forms of ACC typically contain very little molecular water, whereas most stable forms are hydrates commonly having the formula $\text{CaCO}_3 \cdot \text{H}_2\text{O}$.²²⁾ However, the characteristic XRD peak of $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ was not identified during the initial retention time (from 10 to 30 min), compared to previous crystalline information²³⁾ (Appendix Fig. A3). $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ have negative enthalpies of formation with respect to calcite, but are unstable with respect to the same phase at ambient conditions, based on free energy. The entropy change per water is 32.0 for $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ and 33.5 $\text{J K}^{-1} \text{mol}^{-1}$ for $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$.¹⁾ These entropy values indicate that the ACC and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ to

calcite transitions are not thermodynamic in nature. Interactions with additives, kinetic factors, and the competition of $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ need to be considered as well.

A recent study of the biomineralization of sea urchin spicules suggests that hydrated ACC is the likely precursor of the anhydrous form,²⁴⁾ indicating the possibility that hydrated ACC plays a broader role than previously thought and that its transition may occur via multiple steps. In this study, it was demonstrated that serine and arginine play a significant role in the retention of CaCO_3 phases (ACC and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$) and also in the preferred orientation associated with crystal morphology. Although the amino acid impacts the retention of ACC and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$, the crystalline phase transition of ACC to calcite is related to the intrinsic characteristics of CaCO_3 crystallization, controlled by thermodynamic parameters and not the effects of amino acids.

On the other hand, the formation of aragonite, important in shell formation, suggests effects from organic molecules secreted by bivalves and not thermodynamic parameters.

Marine organisms, including bivalves, most likely utilize the effects of activation energy for the control of CaCO_3 polymorphs. For example, bivalves use organic molecules to synthesize aragonite, which is thermodynamically unstable compared to calcite.^{25,26)} Unless the conditions that affect thermodynamic factors, such as temperature, pH value, and supersaturation are induced by an excess of amino acid, ACC can transform to calcite, which is thermodynamically stable and kinetically advantageous. The differences in the solubility^{27,28)} of calcite ($\log K_{\text{sp}} -8.42$) and aragonite ($\log K_{\text{sp}} -8.22$) show that calcite is more stable under aqueous conditions than aragonite.

Due to the development of the main peak (104) (Fig. 1) and knowledge of the shell formation of bivalves, the key component in the calcite transition might be ACC and not $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$, though $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ would also transform to calcite over time. To understand the transition pathway in more detail, both the crystal model of ACC and crystallographic information on the correlation between CaCO_3 and amino acids are necessary.

4. Conclusions

In the synthetic process of CaCO_3 thin films, serine (25 mM) and arginine (25 mM) show an effect on the retention time of amorphous calcium carbonate (ACC) and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$. This factor affects the preferred orientation and the morphology of CaCO_3 thin films synthesized under atmospheric conditions (room temperature and 1 atm). In the absence of amino acids, a burr-like crystal structure with crystal face (202) was identified, and a flower-like structure with crystal faces (006) and (018) was identified in the presence of arginine. ACC and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ to calcite is a solid-state transformation, and ACC and/or $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ are precursors involved in the phase change.

The methodology in this study is a new approach to overcome the limits of studying *in vitro* CaCO_3 crystallization. The data in this study serve to better explain the effect macromolecule additives have on biomineralization, based on the morphological control of CaCO_3 and the interaction between ACC or $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$.

Fig. A1 Simulated X-ray pattern of $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$.

Fig. A2 Result of blank measurement using Si-low background sample holder (Cu tube, 40 kV-40 mA, DS = SS = 1°, RS = 0.15 mm, $2\theta/\theta$ measurement).

Acknowledgements

This research was supported by the General Research Project (Utilization and Sequestration of CO_2 Using Industrial Minerals) of the Korea Institute of Geoscience and Mineral Resources (KIGAM), funded by the Ministry of Knowledge Economy of Korea.

Appendix

Supplementary information shows the simulated X-ray pattern of $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ and $\text{CaCO}_3 \cdot \text{H}_2\text{O}$.

Figure A1 shows XRD pattern of $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$. We simulated the pattern based on the previous information²⁹⁾ by using the software³⁰⁾ (VESTA (Visualization for Electronic and Structural Analysis) and RIETAN). The vertical bar means the Bragg position of X-ray diffraction calculated by RIETAN. The intensity of main peak (17.11°) was assumed to be 100 and all peaks has been normalized. The black arrows mean first and second peak of $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$. We could identify the peaks at the each initial retention time of Fig. 1.

Figure A2 shows the XRD result of blank measurement using Si-low background sample holder. As shown in Fig. A2, the use of the Si-low background sample holder

Fig. A3 Simulated X-ray pattern of $\text{CaCO}_3 \cdot \text{H}_2\text{O}$. The vertical bar means the Bragg position of X-ray diffraction calculated by RIETAN. The intensity of main peak (15.17°) (A3(a)) and (20.47°) (A3(b)) were assumed to be 100 and all peaks has been normalized.

Fig. A4 Schematic illustration for the formation of CaCO_3 film. The photo on the lower righthand side shows silicon wafer coated by CaCO_3 film (scale bar 25 mm).

also produces a very small, broad background attributable to continuous X-rays (DMAX 2200 PV. RIGAKU), although it is much lower than the background produced with an ordinary glass sample holder. Specifications on low background sample holder is as follows;

- (1) Material: Single-crystal silicon for semiconductors
- (2) Cut-out direction: $(911) \pm 0.1^\circ$
- (3) External dimensions: 35 (W) $\times$ 50 (H) $\times$ 2 (T) mm

Figure A3 shows XRD patterns of $\text{CaCO}_3 \cdot \text{H}_2\text{O}$. We simulated the pattern based on the previous information³¹⁾ by using the software³⁰⁾ (VESTA (Visualization for Electronic and Structural Analysis) and RIETAN).

Only $\text{Ca}(\text{OH})_2$ powder and DW (distilled water) were used. Figure A4 shows that CaCO_3 film was synthesized at the interface between solution and air.

REFERENCES

- 1) P. Raiteri and J. D. Gale: *J. Am. Chem. Soc.* **132** (2010) 17623–17634.
- 2) H. Ehrlich: *Biological Materials of Marine Origin*, (Springer, New York, 2010) pp. 25–101.
- 3) A. Lin and M. A. Meyers: *Mater. Sci. Eng. A* **390** (2005) 27–41.
- 4) H. B. Yao, Z. H. Tan, H. Y. Fang and S. H. Yu: *Angew. Chem. Int. Ed.* **49** (2010) 10127–10131.
- 5) J. R. Clarkson, T. J. Price and C. J. Adams: *J. Chem. Soc. Faraday Trans.* **88** (1992) 243–249.
- 6) D. Medakovic, S. Popovic, B. Crzeta, M. Plazonic and M. Hrs-Brenko: *Mar. Biol.* **129** (1997) 615–623.
- 7) C. L. Freeman, J. H. Harding, D. Quigley and P. M. Rodger: *Angew. Chem. Int. Ed.* **49** (2010) 5135–5137.
- 8) D. E. Jacob, R. Wirth, A. L. Soldati, U. Wehrmeister and A. Schreiber: *J. Struct. Biol.* **173** (2011) 241–249.
- 9) S. W. Lee, K. B. Lee and S. B. Park: *Micron* **40** (2009) 737–742.
- 10) A. R. Lennie, C. C. Tang and S. P. Thompson: *Mineral. Mag.* **68** (2004) 135–146.
- 11) J. D. Rodriguez-Blanco, S. Shaw and L. G. Benning: *Nanoscale* **3** (2011) 265–271.
- 12) E. Beniash, J. Aizenberg, L. Addadi and S. Weiner: *Proc. R. Soc. Lond. B* **264** (1997) 461–465.
- 13) L. Addadi and S. Weiner: *Proc. Natl. Acad. Sci. USA* **82** (1985) 4110–4114.
- 14) A. W. Xu, Y. Ma and H. Cölfen: *J. Mater. Chem.* **17** (2007) 415–449.
- 15) S. Weiner and L. Addadi: *Annu. Rev. Mater. Res.* **41** (2011) 21–40.
- 16) E. Loste, R. M. Wilson, R. Seshadri and F. Meldrum: *J. Cryst. Grow.* **254** (2003) 206–218.
- 17) J. Aizenberg, G. Lambert, S. Weiner and L. Addadi: *J. Am. Chem. Soc.*

- 124** (2002) 32–39.
- 18) A. Becker, A. Ziegler and M. Epple: *Dalton Trans.* **10** (2005) 1814–1820.
- 19) S. Ras, S. Weiner and L. Addadi: *Adv. Mater.* **12** (2000) 38–42.
- 20) E. Bonucci: *Biological Calcification*, (Springer-Verlag Berlin Heidelberg, 2010) pp. 23–144.
- 21) A. Sato, S. Nagasaka, K. Furihata, S. Nagata, I. Arai, K. Saruwatari, T. Kogure, S. Sakuda and H. Nagasawa: *Nat. Chem. Biol.* **7** (2011) 197–199.
- 22) A. L. Goodwin, F. M. Michel, B. L. Phillips, D. A. Keen, M. T. Dove and R. J. Reeder: *Chem. Mater.* **23** (2010) 3197–3205.
- 23) H. Effenberger: *Monatshefte für Chemie* **112** (1981) 899–909.
- 24) Y. Politi, R. A. Metzler, M. Abrecht, B. Gilber, F. H. Wilt, I. Sagi, L. Addadi, S. Weiner and P. U. P. A. Gilbert: *Proc. Natl. Acad. Sci. USA* **105** (2008) 17362–17366.
- 25) A. M. Belcher, X. H. Wu, R. J. Christensen, P. K. Hansma, G. D. Stucky and D. E. Mose: *Nature* **381** (1996) 56–58.
- 26) G. Falini, S. Albeck, S. Weiner and L. Addadi: *Science* **271** (1996) 67–69.
- 27) S. Mann: *Biom mineralization: Principles and Concepts in Bioinorganic Materials Chemistry*, (Oxford University Press, New York, 2001) pp. 6–191.
- 28) D. Gebauer, A. Völkel and H. Cölfen: *Science* **322** (2008) 1819–1822.
- 29) A. R. Lennie, C. C. Tang and S. P. Thompson: *Mineral. Mag.* **68** (2004) 135–138.
- 30) K. Momma and F. J. Izumi: *Appl. Crystallogr.* **41** (2008) 653–657.
- 31) H. Effenberger: *Monatshefte für Chemie* **112** (1981) 899–904.