

Reply to “Mirror Symmetry Breaking” of the Centrosymmetric CaCO₃ Crystals with Amino Acids**

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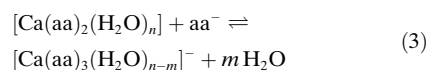
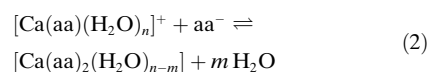
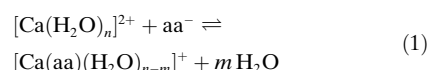
amino acids · biomineralization · calcium carbonate · chirality · homochirality

In their Comment in *Angewandte Chemie*, Lahav and Leiserowitz state that the crystallization experiments reported in the contribution “Phase Selection of Calcium Carbonate through the Chirality of Adsorbed Amino Acids”^[1] violate basic rules of symmetry and thermodynamics. Ruling out insufficient statistics, they suspect the presence of chemical, biological, or other homochiral contaminants^[2] in the system. Lahav and Leiserowitz rest their assumption on etch figures induced by the presence of D- and L- α -amino acids in the growth and morphology of the centrosymmetric α -polymorph of glycine^[3] and their effect on its polymorphic behavior. We present here additional data on the crystallization studies of calcium carbonate in the presence of homochiral amino acids that were not contained in the original paper.

We do not question the basic explanation of the crystallography of the

interaction of chiral molecules with opposite surfaces (hkl) and ($\bar{h}\bar{k}\bar{l}$) of centrosymmetric crystals, irrespective of the surface's plane group symmetry. These symmetry arguments certainly apply to the growth, dissolution, and physical properties of the defect-free extended crystal, but they should also be valid in the presence of potential homochiral contaminants. It is highly debatable, however, whether these arguments apply to the nucleus during the early stages of crystal formation, even prior to phase selection.^[4]

In their crystallization experiments with glycine, Leiserowitz and Lahav view the solution merely as a reservoir of discrete and non-interacting molecular building blocks that are added one by one to the surface of the growing crystal. While this may be a reasonable model for the crystallization of glycine, it is certainly an inappropriate picture for the crystallization of calcium carbonate. Lahav and Leiserowitz overlooked that the amino acids cannot be considered as innocent molecules, because they are involved as ligands in the coordination chemistry of Ca²⁺.^[5] CaCO₃ (bio-)mineralization, that is, nucleation and crystal growth in the presence of amino acids (Haa), peptides, or proteins, is dictated kinetically and thermodynamically by Ca²⁺-amino acid complex formation equilibria such as (1)–(3).



Most of the possible Ca²⁺ complexes have neither been demonstrated in solution nor isolated and structurally characterized. Assuming only six-coordinate metal centers, a large number of enantiomeric and diastereomeric Ca²⁺-amino acid complexes may co-exist (see Figure S2 in the Supporting Information), and it seems plausible that five- or seven-coordinate species exist as well.^[6] These different compounds will exert a distinct influence on crystal growth and dissolution, as they have different structures, symmetries, and thermodynamic stabilities.

Based on the stability and protonation constants given in reference [7], the complex [Ca(Hala)(H₂O)_n]²⁺ is present only in small amounts for pH > 8.5. However, when the solution becomes more basic, the alaninato complex [Ca(ala)(H₂O)_n]⁺ is formed, and at pH values of > 11 reaches a constant concentration corresponding to 16 % of the total amount of dissolved calcium.

The positive-ion ESI mass spectra (Figure 1a) of solutions containing Ca²⁺ and alanine in H₂O is consistent with the discussion above: the solutions contain mono-, di-, and tri-substituted Ca²⁺ alaninato complexes, that is, [Ca(ala)₃ + 2H]⁺, [Ca(ala)₂(H₂O)_x + H]⁺ (0 ≤ x ≤ 2) and [Ca(ala)(H₂O)₂]⁺. Prominent signals at higher masses can be partially assigned to compounds with more than one calcium ion: [Ca₂(ala)₂(H₂O)₅]²⁺, [Ca₂(ala)₃(H₂O)]⁺, [Ca₂(ala)₃(H₂O)₅ + H]⁺, and [Ca₃(ala)₄(H₂O)]²⁺. A detailed discussion of these

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Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

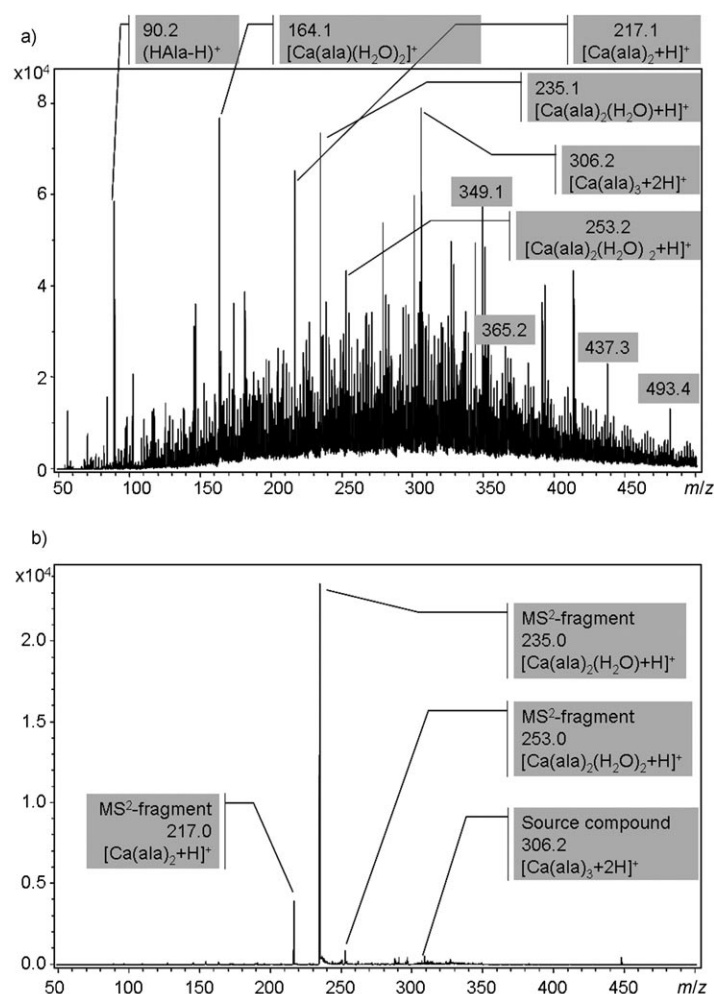


Figure 1. ESI mass spectra of a calcium-alanine solution. a) Full mass spectrum in the range $50 \leq m/z \leq 500$ and b) MS^2 (306 m/z) to show fragment ions during collision of the calcium trisalaninato compound with He^+ ions.

experiments is given in Section 1 in the Supporting Information.

The coordination chemistry of Ca^{2+} in combination with the above mass spectrometric data indicates that possible clues for understanding the observed phase selection of calcium carbonate in the presence of chiral amino acids lie in their complexation behavior in solution. In fact, the pH and $[Ca^{2+}]$ profiles of the crystallizations in Figure 2 reveal significant differences in the presence of D- or L-alanine. For L-alanine we observe a uniform crystallization profile characteristic of a steady precipitation, for D-alanine the precipitation appears to be a two-step process. The observed differences in the solution pH cannot be explained by the presence of chemical, biological, or other homochiral contaminants, because the pH value is a thermodynamic bulk property of the solu-

tion. For a detailed discussion see the Section 2 in the Supporting Information.

We agree with Lahav and Leiserowitz that impurities may play an important role in the early stages of crystal nucleation. Enantiomers of the amino acids are prepared by different synthetic routes and may be contaminated, for example, with synthetic chiral precursors. The commercially available L-amino acids are prepared by fermentation and extraction from natural products. Each amino acid has to be separated from complex mixtures of different amino acids. As a result, commercial L-amino acids may be contaminated by small amounts of other L-amino acids. On the other hand, D-amino acids are synthesized either by asymmetric synthesis or by separation from racemic mixtures that are prepared by racemization of the L-amino acid.

The purity of the D- and L-alanine and D- and L-valine was checked routinely by 1H NMR spectroscopy (see Figures S6–S9 in the Supporting Information). To rule out contaminations of the enantiopure amino acids, their purity was analyzed additionally by gas chromatography using chiral columns. The samples of D- and L-alanine and D- and L-valine contain less than 0.1% of impurities, the samples of D- and L-proline contain 0.13% and 0.12% of impurities (see Table S2 in the Supporting Information). In the case of D- and L-proline, these impurities might possibly be due to other amino acids.

Based on the analytical data one would rule out impurities to be the driving force for the observed phase selection of calcium carbonate. Still, to probe the influence of chiral contaminants on the phase selection in a con-

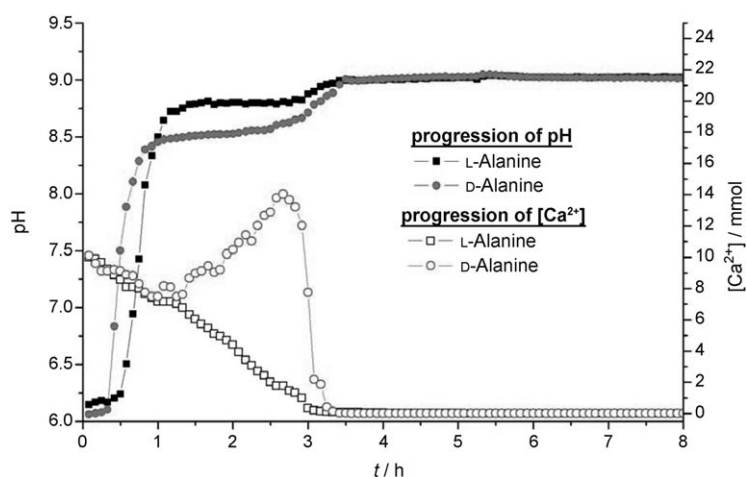


Figure 2. Progression of pH and $[\text{Ca}^{2+}]$ during a crystallization with L- and D-alanine as additives. (The apparent $[\text{Ca}^{2+}]$ in excess of the nominal Ca^{2+} content (10 mmol) of the parent solution is due to the fact that the Ca-sensitive electrode is also sensitive to H^+ and NH_4^+ .)^[11]

trolled fashion, the crystallization of CaCO_3 was carried out in the presence of enantiopure amino acids with and without addition of 1.0% and 1.0% of a second (enantiopure) amino acid, respectively (Figure 3). The phase analysis of the precipitates shows that the phase selectivity in the presence of homochiral amino acids, which was reported in reference [1], has completely vanished already at an impurity level of 1% (see Table S1 and Figure S5 in the Supporting Information). A detailed discussion and phase analysis by means

of X-ray diffraction data are given in Section 3 in the Supporting Information. Thus, homochiral impurities that were claimed by Lahav and Leiserowitz^[8] to be the driving force of the observations reported in reference [1] clearly eliminate the effect of phase selection rather than promote it.

It is a well-known fact that the polymorphism of calcium carbonate is affected by other factors such as solution pH, temperature, supersaturation, and initial ion concentrations, the solution pH being an important factor. At ambient

temperature and in the absence of additives, vaterite was reported to be the major product in the pH range between 8.5 and 10.^[9] The yields of aragonite and calcite increase with increasing pH val-

ue. Aragonite formation shows a maximum at pH 11, above pH 12 calcite is the dominant product.^[10] When CaCO_3 is precipitated from $\text{Ca}(\text{OH})_2$ solutions in a CO_2 atmosphere in the presence of enantiopure amino acid additives following a procedure given by Page and Cölfen,^[11] only calcite was formed (Figure 4 and S11 in the Supporting Information).

The difference between the mineralization experiments following the ammonium carbonate method and the Cölfen method^[11] lies in the starting pH value and the pH evolution during the precipitation process (see Figure S12 in the Supporting Information). The pH value exerts not only a strong influence on the supersaturation level by controlling the CO_3^{2-} and HCO_3^- concentrations, it determines also—through the protonation equilibria of the amino acid (Haa , aa^- [zwitterionic], or aa^-)—the concentration of the active Ca^{2+} solution species that is involved in the complex-

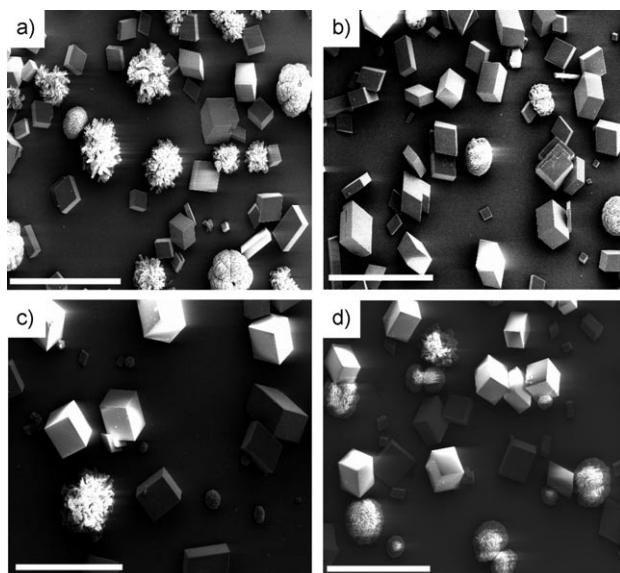


Figure 3. SEM images of calcium carbonate crystallized in the presence of enantiopure L- and D-alanine. a) L-alanine with 1.0% L-valine, b) L-alanine with 1.0% D-valine, c) D-alanine with 1.0% L-valine, and d) D-alanine with 1.0% D-valine. Scale bars: 200 μm .

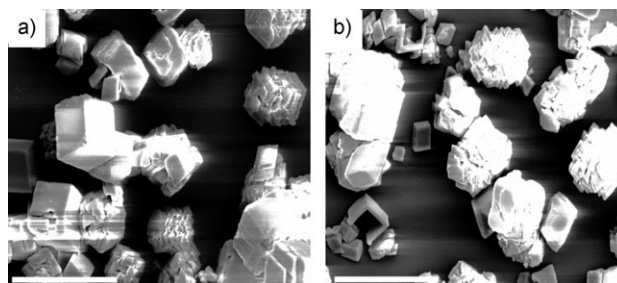


Figure 4. SEM images of calcium carbonate crystallized in the presence of enantiopure valine from $\text{Ca}(\text{OH})_2$ under CO_2 atmosphere: a) L-valine and b) D-valine. Scale bars: 20 μm .

ation equilibria given by Equations (1), (2), and (3).

In summary, different from the crystallization of glycine, the nucleation and growth of CaCO_3 in the presence of amino acids is determined to a large extent by complex formation equilibria that involve the amino acids and Ca^{2+} . The existence of mono-, di-, and trisubstituted Ca^{2+} amino acid complexes has been demonstrated by ESI mass spectrometry. For bifunctional chiral ligands a large number of enantiomeric and diastereomeric complexes can be formed, which must be regarded as “intrinsic homochiral contaminants”. Chiral impurities that were claimed by Lahav and Leiserowitz^[8] to be the source of the observed phase selectiv-

ities in reference [1] show exactly the opposite of the proposed effect: *When added intentionally, they annihilate the observed phase selectivity rather than promoting it.*

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