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Biomineralization: Systematics of organic-directed controls on carbonate growth morphologies and kinetics determined by *in situ* AFM
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I. Summary of Major Findings

During the three years of this project, Professor Dove's laboratory has made tremendous progress in understanding the microscopic kinetic controls on calcite growth and in investigations of amino acid controls on modifying crystal growth and dissolution. Dove and students, Mr. Henry Teng, Mr. J. Zhao and Mr. K. Davis (new student) began the project with a focus on the aspartate-calcite system because previous studies have found that acidic matrix macromolecules involved in the regulation of biological crystal growth usually contain aspartic acid-rich domains (Crenshaw, 1972; Weiner *et al.*, 1983; Weiner, 1986; Addadi *et al.*, 1987; Greenfield and Crenshaw, 1990). Indeed, several studies have shown that aspartate (Asp) modifies the growth morphology of calcite (e.g. Addadi and Weiner, 1985; Addadi *et al.*, 1987; Mann *et al.*, 1990). Aspartate-rich proteins and β -sheet polyaspartate adsorbed on sulfonated polystyrene surfaces were shown to stabilize {0001} growth surfaces. It was also shown that aspartate also stabilizes the prismatic {1 $\bar{1}$ 00} growth forms (Berman *et al.*, 1993, 1995). This suggests that systematic relationships between crystallization controls and surface interactions with the reactive groups of organic molecules must exist. However, these studies addressed growth processes through *ex situ* macroscopic approach. For the first time, we have an understanding of the microscopic controls of aspartate on growth and dissolution.

Phase 1: Methods development

In this important initial stage of the project, we developed the experimental procedures needed to examine *in situ* growth on seeded substrates including methods to conduct constant composition, flow-through AFM observations of steady-state mineral growth and dissolution. Upon developing these methods, we made new findings leading to several manuscripts and a M.S. Thesis and an upcoming Doctoral dissertation. Some of our findings to date are summarized below.

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Phase 2: Surface Site-Specific Interactions of Aspartate with Calcite During Dissolution: Implications for Biomineralization
(American Mineralogist, 1997) — Reprint

Calcite occurs widely as a mineral component in the exoskeletons and tissues of marine and freshwater invertebrates. Matrix macromolecules involved in regulating the biological growth of calcite in these organisms are known to share a carboxylic-rich character that arises from an abundance of the acidic amino acids, aspartate (Asp) and glutamate (Glu). This study determines the interactions of Asp with calcite $\{10\bar{1}4\}$ faces during dissolution using in situ Fluid Cell Atomic Force Microscopy (AFM) and macroscopic ex situ optical methods. In control experiments, etch pit morphologies produced by dissolution in simple undersaturated solutions reflect the inherent symmetry of the $\{10\bar{1}4\}$ faces with a rhombus form. With the introduction of Asp, surface site reactivities are modified to yield isosceles triangular etch pits and hillocks. With continued exposure to Asp-bearing solutions, these triangular pits coalesce and the surface evolves into a network of interconnected tetrahedral etch hillocks. The component tetrahedral 'sides' have Miller-Bravais indices of (0001) , $(1\bar{1}01)$, and $(01\bar{1}1)$. These faces intersect the $(10\bar{1}4)$ face in the $[010]$, $[45\bar{1}]$ and $[\bar{4}11]$ directions to comprise the three edges of the triangular etch pits. Structural and stereochemical constraints suggest that the $(1\bar{1}01)$ and $(01\bar{1}1)$ faces in the hillock are a combination of corresponding faces from the $\{1\bar{1}02\}$ and $\{1\bar{1}00\}$ crystallographic forms.

Phase 3: An AFM study of Calcite Dissolution in Selected Amino Acids
(M.S. Thesis, 1997)

The formation of calcite through biomineralization processes usually involves aspartate and glutamate-rich proteins, which serve as substrates for the nucleation and growth of calcite. In this study the interactions between selected amino acids and the calcite $(10\bar{1}4)$ face during dissolution were investigated by both *in situ* fluid cell atomic force microscopy (AFM) and *ex situ* optical methods. AFM experiments were conducted under various conditions by batch and flow-through methods. The objective of the study was to determine systematic relationships among the functional groups of amino acids and the etch pit morphology of calcite developed in corresponding amino acid solutions.

Acidic amino acids (Asp and Glu) and an amino acid with a polar side chain (Asn) have the most profound modifying effects, while a basic amino acid (Lys) and an amino acid with a hydrophobic side chain (Leu) did not show any modifying effects. Both AFM observations and etching experiments showed that etch pits in glutamate (Glu) solutions developed two new step edges with Miller-Bravais indices $[45\bar{1}]$ and $[\bar{4}11]$, resulting from the stabilization of the same $(\bar{1}101)$ and $(0\bar{1}11)$ faces as are stabilized in aspartate (Asp) solutions. However, (0001) faces, which formed in Asp solutions, did not develop in Glu solutions. Long-term dissolution in asparagine (Asn) solutions produced triangular pits with three new step edge

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directions, while pit morphology observed by AFM was characterized by a rhombic pit with one acute corner curved.

The control of functional groups on pit morphology results from the selective adsorption of amino acids by calcite and the interactions between side-chain functional groups and >Ca surface sites with specific geometries. Calcite can selectively adsorb Asp, Glu and Asn. The functional groups in these three amino acids mimic carbonate groups in the calcite structure, allowing molecular recognition to occur on specific calcite surface sites. Amino acids with hydrophobic side chains and basic side chains did not show this type of specific interaction. The different reactivities of surface sites are responsible for the anisotropy of dissolution of calcite in both deionized water (DIW) and amino acid solutions.

The pH dependence of etch pits developed in Glu solution results from the differences in degree of Glu adsorption by calcite at various pH. The discrepancy of the etch pit morphology of calcite observed by AFM in some amino acid solutions from those in corresponding long-term experiments suggests that the use of AFM as a tool in real-time biomineralization study will require careful selection of experimental conditions.

To learn more about the physics of crystal growth driving these phenomena, Mr. Teng studied as a Summer Intern with J.J. De Yoreo's group at LLNL during Summer 1996. Our initial collaboration yielded exciting new findings and led us to build a DOE-BES-supported formal collaboration. The following projects have developed from our interdisciplinary efforts.

Phase 4: Reversing Calcite Growth Anisotropy and Morphology Through Perturbations of Microscopic Kinetics - *Preprint* (Nature, submitted)

Recent advances in our ability to unravel the kinetics and mechanisms of crystallization by direct observations have rejuvenated cross-disciplinary interests in carbonate mineralization, particularly, in biological systems where organisms synthesize minerals at very near equilibrium conditions. Inorganic and organic compounds are believed to play various roles as impurities in the processes of biomineralization, but their effect on growth kinetics and resultant growth morphology is not understood. To date, most studies of calcite growth have been limited to the inference of rates and mechanisms by indirect methods that monitor changes in bulk solution chemistry or *ex situ* examinations of growth hillocks, the products of step assembly, at mesoscales. Few studies have directly observed calcite growth at microscopic scales. Here we quantify relationships between solution supersaturation and step migration rates using *in situ* Fluid Cell Atomic Force Microscopy (AFM). Our *in situ* study is the first to show how rates of growth that are highly specific to crystallographic direction can be controlled to produce unique growth geometries through perturbations in microscopic kinetics. For this anisotropic material, we find a complex behavior for the step migration velocities, v_{s+} and v_{s-} , along the conventional 'fast' and 'slow' crystallographic directions,

($[\bar{4}41]_+$ and $[48\bar{1}]_+$, and $[\bar{4}41]_-$ and $[48\bar{1}]_-$, respectively). As solute activity, a , decreases toward its equilibrium value, a_e , v_{s+} and v_{s-} approach each other and become equal when $(a-a_e) \approx 3 \times 10^{-5}$. At lower values of $(a-a_e)$, a reversal in the behavior of step migration velocities causes growth hillocks to assume a novel reversed geometry. Comparisons of our kinetic data with classical theoretical models suggest that these changes may be due to minute impurities that impact rates of growth. At very near-equilibrium conditions, a kinetic 'dead zone' is imposed on positive steps while negative steps continue to migrate at a modified rate. This behavior is analogous to the effect we also find for aspartate, a recognized growth modifier and the most abundant amino acid in macromolecules associated with active biomineralization. Our findings demonstrate the site selective nature of impurities and their ability to direct the development of unusual hillock morphologies at the near equilibrium conditions of many carbonate growth environments.

Phase 5: Thermodynamic Controls on Calcite Mineralization
(Science, in advanced preparation)

Preprint

see attached summary

Phase 6: The Kinetics and Mechanisms of Calcite Growth at Near Equilibrium Conditions
(*Geochimica Cosmochimica Acta*, in advanced preparation)

Microscopic mechanisms and kinetics of calcite crystallization at near equilibrium conditions have been characterized using in situ Fluid Cell Atomic Force Microscopy. Seeded calcite was grown in a series of solutions with known supersaturations and two distinct mechanisms, heterogeneous surface nucleation, in particular, spiral growth, and homogeneous surface nucleation, were observed on the $\{10\bar{1}4\}$ faces upon changing the degree of supersaturation. The critical supersaturation range for the transition was determined. The rates of step migration and the slopes of growth hillocks have been measured on growth spirals formed by screw dislocations. These measurements were used to determine the dependence of crystallographic-specific step migration rates and resultant growth anisotropy upon supersaturations. The results indicate that step migration rates along positive directions, e.g. $[\bar{4}41]_+$ and $[48\bar{1}]_+$, and negative directions, e.g. $[\bar{4}41]_-$ and $[48\bar{1}]_-$ inverted at approximately $\sigma=0.2$ which leads to a reversal of hillock geometry as well. These observations and the non-linear dependence of step migration rates upon σ at very near equilibrium conditions suggest that growth kinetics are crystallographic direction-specific. These findings from this microscopic study of crystallization in simple $\text{Ca-CO}_2\text{-H}_2\text{O}$ system lead us to further predict that other anisotropic minerals and materials may behave in a similar way because sites with different reactivities are also likely to occur along different crystallographic directions.

Phase 7a: Investigation of Bacterial-Mineral Interactions Using Fluid Cell Atomic Force Microscopy (Geochimica Cosmochimica Acta, 1996) - Reprint

An unanticipated study of microbe-mineral surface interactions was also yielded as an indirect result of this biomineralization study. The two publications that came from this work are summarized in 7a and 7b.

Adhesion of bacteria to the porous media of subsurface environments and their mobility through these systems are of concern for *in situ* bioremediation and infectious disease control. Dissimilatory reducing bacteria can be major contributors to both good and poor water quality due to their ability to catalyze sulfate and iron reduction as well as denitrification reactions. Recent advances in Fluid Cell Tapping Mode™ Atomic Force Microscopy (Fluid TMAFM) make it possible to better understand these natural phenomena through direct observations of *live* bacteria interactions with mineral surfaces. In this study, Fluid TMAFM was used to investigate the effect of iron coatings on the interactions of *Shewanella putrefaciens* with a silica surface (as an analog for quartz). *S. putrefaciens* are facultative anaerobic dissimilatory iron-reducing bacteria that are closely related to *Pseudomonas* spp. These were seeded onto iron-coated and uncoated silica glass substrates in aqueous solutions of varying nutrient composition and incubated under aerobic conditions for 1-4 days. Seeded and control samples were examined using Air and Fluid TMAFM. Observations of live bacteria-surface interactions found that bacteria seeded in nutrient-depleted solutions adhered to iron-coated substrates more strongly than bacteria seeded in liquid growth media. Also, they adhered more strongly to iron-coated than uncoated silica glass. Bacteria seeded in liquid growth media were mostly flagellated suggesting that motility and adhesion are related. Under some conditions the bacteria create pits and eroded areas in the iron coating within 3 days. The mechanism for this is currently unknown. These findings suggest that adhesion, motility, and iron surface chemistry are interrelated in subsurface environments where iron-reducing microorganisms are present.

Phase 7b: Microbially-catalyzed Dissolution of Iron and Aluminum Oxyhydroxide Mineral Coatings (Geochimica Cosmochimica Acta, 1997) - Reprint

This experimental study investigated the processes by which microbes interact with oxyhydroxide mineral surface coatings using an approach designed to better represent the conditions of natural subsurface environments. The interactions of *Shewanella putrefaciens*, a facultative anaerobe capable of dissimilatory iron reduction, with coatings of Fe^{3+} and Al^{3+} oxyhydroxides on natural quartz and silica glass surfaces were examined. Using synthetic groundwater solutions having compositions that simulated a typical aquifer, bacteria were seeded onto mineral surfaces (and coatings) and incubated in parallel with abiotic controls for up to 96 hours under aerobic and anaerobic conditions. Microbial-mineral surface interactions were determined using the direct observational technique, Fluid

Tapping Mode™ Atomic Force Microscopy (TMAFM) in combination with measurements of ferrous iron concentrations and pH of the incubating solutions.

Observations of live bacteria-surface interactions exposed to aerobic conditions found localized pitting on Fe^{3+} oxyhydroxide-coated quartz surfaces within 72 hours of incubation. These pits directly corresponded to sites of bacterial surface adhesion and the extent of pitting was accompanied by the accumulation of ferrous iron to low but steady state concentrations. Localized pitting was not observed on any Al^{3+} oxyhydroxide-coated surfaces. In contrast, iron coated surfaces exposed to bacteria under anaerobic conditions revealed progressive, non-localized iron loss over a period of 96 hours. This correlated with a temporal increase in ferrous iron concentrations in the bacteria-exposed solutions compared to the abiotic controls.

Aqueous chemical measurements combined with the Fluid TMAFM observations indicate biologically-catalyzed iron reduction under both aerobic and anaerobic incubation. We propose that the 'pitting' mechanism observed under aerobic conditions is the result of a redox reaction at the bacteria-iron interface followed by the reoxidation of Fe^{2+} onto the surface. The evidence suggests that bacteria under anaerobic conditions maximize rates of dissimilatory reduction by remaining passively mobile on the surface. This decreased bacterial adhesion under anaerobic conditions enhances opportunities for bacteria-iron mineral surface contact. Results of this study may improve our understanding of relationships between the redox environment and bacterial mobility in the subsurface.

II. Listing of Publications, Thesis, and Conference Abstracts

Refereed Publications

Teng, H. and P.M. Dove (1997) Crystal chemical controls of aspartate in the surface-directed dissolution of calcite: Implications for biomineralization. *American Mineralogist* 82, p. 878-887.

Grantham, M.C. and Dove, P.M. and T.J. DiChristina (1997) Biogeochemistry of subsurface environments: Investigation of bacterial effects on iron and aluminum oxyhydroxide coatings. for *Geochimica et Cosmochimica Acta*. 61, p.4467-4477.

Grantham, M.E. and P.M. Dove (1996) Bacterial-mineral surface interactions: Investigations using Fluid Tapping Mode Atomic Force Microscopy. *Geochimica et Cosmochimica Acta*, 60, p. 2473-2480.

Teng, H.H., P.M. Dove and J.J. DeYoreo (submitted) Reversal of calcite growth anisotropy near equilibrium. to *Nature*. -Preprint

Teng, H.H., P.M. Dove and J.J. DeYoreo (in advanced preparation) Thermodynamic controls on CaCO_3 mineralization. to *Science*. -Preprint

Teng, H. and Dove, P.M. (in advanced preparation) Microscopic characterization of mechanisms and fundamental parameters governing calcite mineralization. for *Geochimica Cosmochimica Acta*.

Dove, P.M., K.J. Davis, H.H. Teng (in preparation) Systematics of calcite-modified growth by selected amino acids. for *Geochimica Cosmochimica Acta*.

Theses and Dissertations

- Zhao, Y.J. (1997) AFM Study of Calcite Dissolution in Water and Selected Amino Acids. *M.S. Thesis*, Georgia Institute of Technology, Atlanta.
- Teng, H. (in preparation) Calcite: Investigations of the Physical Basis of Biomineralization. *Ph.D. Dissertation*, Georgia Institute of Technology, Atlanta.

Conference Abstracts

- Teng, H., J.J. De Yoreo, **P.M. Dove** and C. Orme (1998) Physical controls on calcite mineralization. *International Mineralogical Association Meeting*, Toronto, August.
- Teng, H. and **P.M. Dove** (1988) The kinetics and mechanisms of calcite growth at near-equilibrium conditions. *International Mineralogical Association Meeting*, Toronto, August.
- DeYoreo, J.J., P. Levine, C. Orme, H. Teng and **P. Dove** (1998) Physical controls on crystal growth during biomineralization. *International Conference on Crystal Growth*, Jerusalem, August.
- Orme, C. H.H. Teng, **P.M. Dove** and J.J. De Yoreo (1997) In situ atomic force microscopy studies of surface morphology and kinetics during growth of CaCO_3 , *American Conference on Crystal Growth West*, Fallen Leaf Lake, CA.
- P.M. Dove**, H.H. Teng and Y.J. Zhao (1997) Unraveling the physical basis of biomineralization. *International V.M. Goldschmidt Conference*, Tuscon.
- Teng, H.H. and **P.M. Dove** (1997) Microscopic characterization of mechanisms and fundamental parameters governing calcite crystallization. *International V.M. Goldschmidt Conference*, Tuscon.
- Dove, P.M.**, M.C. Grantham, and T.J. DiChristina (1997) Influence of iron-reducing microbes on iron oxyhydroxide coatings in aerobic and anerobic environments. *Geological Society of Ameria National Meeting*, October, Salt Lake City.
- Grantham, and **Dove, P.M.** and T.J. DiChristina (1997) Influence of iron-reducing microbes on iron oxyhydroxide coatings in aerobic and anaerobic environments. *Symposium in honor George A. Parks, American Chemical Society*, San Francisco.
- Teng and **Dove, P.M.** (1996) Crystal Chemical controls of aspartate in the site-specific dissolution of calcite: Implications for biomineralization. *Geological Soc. of America National Meeting*, October.
- Grantham and **Dove, P.M.** and T.J. DiChristina (1996) Biogeochemistry of anaerobic subsurface environments: Mobilization of iron oxyhydroxide cements. *Geological Soc. of America National Meeting*, October.
- Grantham, M. and **Dove, P.M.** (1996) Biogeochemistry of subsurface environments: Investigation of bacterial effects on iron oxyhydroxide coatings by Fluid Tapping Mode Atomic Force Microscopy. *V.M. Goldschmidt Conf*, Heidelberg.
- Dove, P.M.** (1995) Biomineralization: Morphological directed growth of carbonates by functional organic molecules. *Earth Materials: Theory, Simulation and Experiment*, Argonne National Laboratory, Chicago.
- Grantham, M. and **Dove, P.M.** (1995) Biogeochemistry of quartz in weathering environments: Modifying effects of bacteria on iron oxyhydroxide coatings and

cements. *Symposium: Silicate Mineral Weathering, Geological Society of America, New Orleans, 27*, p. A-185

Teng, H. and **Dove, P.M.** (1995) Modifying effects of amino acids on calcite dissolution and crystallization: In situ investigation by atomic force microscopy. *Geological Society of America, New Orleans, 27*, p. A-198.

III. Awards and Honors

In 1996, P.I. Patricia Dove was awarded the Clarke Medal in Geochemistry. This is the highest honor given by the international Geochemical Society for innovative and creative scientific contributions. She was recognized for research activities in mineral-water interactions. In particular, the kinetics of mineral surface chemistry in carbonate (calcite) and silica systems.

Also during 1996, Prof. Dove received a teaching excellence award from the Georgia Tech CETL (Center for Excellence in Teaching in Learning).