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MP Dvorscak

Mark P. Dvorscak

(301) 252-2393

E-mail: mark.dvorscak@eh.doe.gov

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Date

Investigating the Physical Basis of Biomineralization

P.I. Patricia M. Dove, Associate Professor
School of Earth and Atmospheric Sciences, Georgia Institute of Technology
Atlanta, Georgia 30332, (ph: 540.231.2444)
(email: dove@vt.edu)

I. Summary of Major Findings from Research Activities

During the three years of this project, Professor Dove's laboratory made tremendous progress in understanding fundamental controls on crystal growth in simple model systems for the complex phenomenon of biological mineralization. Our collaboration with J.J. DeYoreo was productive and we surpassed the goals set forth in the original proposal to establish a new quantitative understanding of carbonate mineral crystallization. The findings from this project have been widely recognized across the scientific community by the award of the Mineralogical Society of America best paper award in 1998 and the Best University Research Award of 1999 at the Basic Energy Sciences, Division of Geosciences "Interfacial Processes Symposium". In addition, two students working on this project received six different awards for their research findings. The following publications have been released to date:

Publications to-date:

- Orme, C.A., A. Noy, A. Wierzbicki, M.T. McBride, M. Grantham, H.H. Teng, P.M. Dove and J.J. DeYoreo (2001) Selective binding of amino acids to the atomic steps of calcite creates chiral structures. Accepted for *Nature*.
- Davis, K.J., P.M. Dove, and J.J. DeYoreo (2000) The role of magnesium as an impurity in calcite growth. *Science*, 290, p. 1134-1137.
- Teng, H.H., P.M. Dove, and J.J. DeYoreo (2000) Kinetics of calcite growth: Surface processes and relationships to macroscopic rate laws. *Geochimica et Cosmochimica Acta*. 64, p. 2255-2266.
- Wawersik, W.R., J.W. Rudnicki, P. Dove, J. Harris, J.M. Logan, L. Pyrak-Nolte, F.M. Orr, Jr., P.J. Ortoleva, F. Richter, N. R. Warpinski, J.L. Wilson, T.F. Wong (2001) Terrestrial sequestration of CO₂: An assessment of research needs. *Advances in Geophysics*. 43, p. 97-177.
- Teng, H.H., P.M. Dove, and J.J. DeYoreo (1999) Reversed calcium carbonate morphologies induced by microscopic growth kinetics: Insight into biomineralization. *Geochimica et Cosmochimica Acta*. 63, p. 2507-2512.
- Teng, H., P.M. Dove, C.A. Orme and J.J. DeYoreo (1998) Thermodynamics of calcite growth: Baseline for understanding biomineral formation. *Science*, 274, p. 724-727.

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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. (received MSA Best Paper Award)

Publications submitted or in advanced preparation:

Teng, H., P.M. Dove, and J.J. DeYoreo (near submission) Kinetic and thermodynamic impacts of aspartic acid on calcite crystallization. for *Journal of Crystal Growth*.

Davis, K.J., P.M. Dove, and J.J. DeYoreo (2000) Molecular-scale basis for Mg-calcite crystal morphologies. for *Nature*, 290.

Grantham, M.C., Davis, K.J., P.M. Dove and J.J. DeYoreo (1999) Controls of amino acid functional group chemistry on calcite growth: Determining foundations of biomineral formation. For *Geochimica Cosmochimica Acta*.

Theses and Dissertations Accepted:

Teng, H.H. Ph.D. Degree. *The Thermodynamics and Kinetics of Calcite Crystallization: Baseline for Understanding Biomineral Formation*. Georgia Institute of Technology. Completed May 1999

Zhang, Y.J. M.S. Degree. *An AFM Study of Calcite Dissolution in Water and Selected Amino Acids*. Georgia Institute of Technology. Completed August 1998

Kevin Davis. M.S. Degree. *Magnesium as an impurity in calcite growth: Thermodynamic and kinetic controls on biomineral formation*. Georgia Institute of Technology. Completed May 2000 and is presently a Ph.D. student in my research group at Virginia Tech.

Extended abstracts and special conferences:

Teng, H.H., P.M. Dove, J.J. De Yoreo (in press) The kinetics of calcite growth: Interpreting chemical affinity-based rate laws through the lens of direct observation. In *Morphology and Dynamics of Crystal Surfaces in Complex Molecular Systems*. Materials Research Society Symposium Series. Ed. J.J. De Yoreo.

Davis, K.J., Dove, P.M. and De Yoreo, J.J. (in press) Resolving the Control of Magnesium on Calcite Growth: Thermodynamic and Kinetic Consequences of Impurity Incorporation for Biomineral Formation. In *Morphology and Dynamics of Crystal Surfaces in Complex Molecular Systems*. Materials Research Society Symposium Series. Ed. J.J. De Yoreo.

Conference Abstracts

Davis, K.J., Dove, P.M. and De Yoreo, J.J. (2001) Molecular-Scale Mechanism for Altered Calcite Morphologies in the Presence of Magnesium: Implications for Impurity Modification of Anisotropic Materials. *Spring 2001 Meeting of the Materials Research Society*.

Davis, K.J., Dove, P.M. and De Yoreo, J.J. (2001) Mechanisms of Calcite Growth Inhibition by Mg^{2+} and Sr^{2+} : Comparison of Molecular-Scale Measurements to Crystal Growth Impurity Models. *11th Annual V.M. Goldschmidt Conference, Hot Springs, VA*.

- Davis, K.J., Dove, P.M. and De Yoreo, J.J. (2001) Resolving the Role of Mg^{2+} as an Impurity in Calcite Growth. *11th Annual V.M. Goldschmidt Conference, Hot Springs, VA.*
- Davis, K.J., P.M. Dove, and J.J. DeYoreo (2000) Understanding the Molecular-Scale Processes of Mineralization: The Role of Mg^{2+} in Calcite Growth. *American Geophysical Union, San Francisco, December.*
- Orme, C.A., J.J. DeYoreo, A. Noy, M.T. McBride, A. Wierzbicki, M. Grantham, P.M. Dove (2000) Selective Binding of Chiral Amino Acids to the Atomic Steps of Calcite: Making the Case for Energetic Controls During Biomineralization. *American Geophysical Union, San Francisco, December.*
- Davis, K.J., P.M. Dove, and J.J. DeYoreo (2000) Control of magnesium on calcite growth: Kinetic and thermodynamic consequences of impurity incorporation on biomineral formation. *Materials Research Society, San Francisco, April.*
- Dove, P.M., H.H. Teng and J.J. DeYoreo (2000) The kinetics of calcite growth: Re-interpreting chemical affinity based rate laws through the lens of direct observations of surface processes. *Materials Research Society, San Francisco, April.*
- Davis, K.J., P.M. Dove and J.J. DeYoreo (1999) Magnesium as an impurity on calcite growth: A model system for the incorporation of inorganic impurities into biominerals. Geological Society of America Annual Conference, Denver, October.
- Teng, H.H., P.M. Dove and J.J. DeYoreo (1999) Calcite growth kinetics: In situ observations of surface processes and relationships to affinity-based rate laws. Geological Society of America Annual Conference, Denver, October.
- Grantham, M.C., Davis, K.J., P.M. Dove and J.J. DeYoreo (1999) Effect of organic compounds on calcite growth: Determining foundations of biomineral formation. Geological Society of America Annual Conference, Denver, October.
- Davis, K.J., P.M. Dove and J.J. DeYoreo (1999) The effect of magnesium as an impurity on calcite growth: A model system for biomineralization. V.M. Goldschmidt International Conference, Cambridge, August.
- Teng, H.H., P.M. Dove and J.J. DeYoreo (1999) The fundamentals of calcite mineralization: Surface processes of spiral growth. V.M. Goldschmidt International Conference, Cambridge, August.
- Teng, H.H., P.M. Dove and J.J. DeYoreo (1999) Kinetics of calcite growth: Analysis of surface processes and relationships to macroscopic rate laws. V.M. Goldschmidt International Conference, Cambridge, August.
- DeYoreo, Teng, Land, Dove et al. (1999) Centennial Meeting of the American Physics Society, Atlanta, March.
- Teng, H.H., P.M. Dove and J.J. DeYoreo (1999) An insitu AFM investigation of the effect of aspartic acid on the energetic and kinetic factors controlling the growth of calcite. Materials Research Society Symposium on Biomineralization, Boston, November.
- Teng, H.H., P.M. Dove and J.J. DeYoreo (1998) Kinetics of near-equilibrium calcite growth: Towards the fundamentals of biomineralization. *Geological Soc. of America National Meeting, Toronto, October.*
- Teng, H.H., P.M. Dove, J.J. DeYoreo and C. Orme (1998) Thermodynamic controls on calcite growth: Determining the foundations of biomineralization. *Geological Soc. of America National Meeting, Toronto, October.*
- Teng, H., C. Orme, P.M. Dove and J.J. De Yoreo (1998) Surface dynamics during $CaCO_3$ homoepitaxy determined by in situ AFM. Materials Research Soc. Annual Meeting, Boston, November.

- De Yoreo, J.J., T. A. Land, C. Orme, H.H. Teng, M. T. Mc Bride, T. Martin, P. M. Dove and G.T. R. Palmore (1998) Trends in surface dynamics during near-equilibrium growth of crystal surfaces by low temperature liquid phase epitaxy. Materials Research Soc. Annual Meeting, Boston, November.
- Teng, H., J.J. De Yoreo, P.M. Dove and C. Orme (1998) Physical controls on calcite mineralization. *International Mineralogical Association Meeting*, p. A91, Toronto, August.
- Teng, H. and P.M. Dove (1998) The kinetics and mechanisms of calcite growth at near-equilibrium conditions. *International Mineralogical Association Meeting*, p. A82, 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.

Junior and Senior Scientists supported by this project:

Undergraduates:	Ms. Bridget Bruton
	Ms. Micki Cook
Graduate Students:	Ms. Meg Grantham
	Mr. Kevin Davis
	Dr. Henry Teng
	(now Assistant Professor at George Washington University)
	Mr. James Zhang
Research Scientist:	Ms. Meg Grantham
Assistant Professor:	Dr. Patricia Dove (now Associate)

Statement of funds that remained at the end of this project:

Our research activities expended all of the funds in this project.

II. Awards and Honors

Patricia Dove
Spring 1996.

Geochemical Society.

Clarke Medal

Fall 1996.

Teaching Excellence Award.

Georgia Institute of Technology

Spring 1998

Best University Research Award, DOE

Interfacial Processes' Symposium

Fall 1998

Best Paper Award

Mineralogical Society of America

Fall 2000

Elected Fellow

Mineralogical Society of America

H. Henry Teng
October 1998

Best Paper Award,

Mineralogical Society of America

April 1999

Dean's Fellowship for Best in Research Award

Georgia Institute of Technology

May 1999

Earth and Atmospheric Sciences Best Dissertation Award

Georgia Institute of Technology

Kevin Davis
April 2000

Gold Award, Graduate Student Research

Materials Research Society (MRS)

San Francisco

February 2001

Sigma Xi, Best Master's Thesis Award

Georgia Tech

March 2001

17th Annual Research Symposium, Physical Sciences

First Place Award

Virginia Tech

III. Summary of Major Findings from Research Activities

1. Reversed Calcite Morphologies Induced by Microscopic Kinetics: Insights into Biomineralization (Teng, Dove, DeYoreo, 1999, *Geochim. et Cosmochim. Acta*). This

experimental investigation of calcite growth quantified relationships between solution supersaturation and rates of step advancement. Using *in situ* Fluid Cell Atomic Force Microscopy (AFM), near-equilibrium growth rates were quantified by measuring the movement of monomolecular steps that comprise growth hillocks on {104} faces. Growth of this anisotropic material exhibited a

complex dependence of step migration rates, v_{S+} and v_{S-} , upon small differences in solution chemistry along the conventional 'fast' ($[\bar{4}41]_+$, $[48\bar{1}]_+$) and 'slow' ($[\bar{4}41]_-$, $[48\bar{1}]_-$) crystallographic directions (Fig. 1). In quantifying the sensitivity of step growth kinetics to supersaturation, we produced growth hillocks with unique geometries (Fig. 2). As supersaturation decreased, v_{S+} and v_{S-} converged and growth hillocks

expressed a pseudo-isotropic form (2AB). At lower supersaturations approaching equilibrium, an inversion in the rates of step advancement led to hillocks with unusual reversed structures (2C). Comparisons of the kinetic data with classical theoretical models suggested that the observed behavior was due to interaction of impurities with the two step types via two different mechanisms: incorporation at the $[\bar{4}41]_+$, $[48\bar{1}]_+$ steps, and blocking by short-lived adsorbates on the $[\bar{4}41]_-$, $[48\bar{1}]_-$ steps. These findings demonstrate the control of crystallographic structure over the local kinetics of growth to stabilize the formation of unusual hillock morphologies that may occur at the near-equilibrium conditions found in natural environments.

2. Thermodynamic Controls on Calcite Mineralization (Teng, Dove, DeYoreo, Orme, 1998, *Science*). The complexity of biomineralized structures suggests the potential of organic constituents for controlling energetic factors during calcification. Using *in situ* AFM to investigate the thermodynamic controls on carbonate growth, we investigated the nature of organic-calcite surface interactions. First, we directly measured the supersaturation dependence of the critical step length, L_c , at the apex of growth

Figure 3

hillocks (Fig 3). Using precisely controlled solution compositions, we showed that this parameter is inversely dependent upon supersaturation, σ , providing in the first experimental confirmation of the classic Gibbs-Thomson (G-T) relationship for any mineral or crystal system (Fig. 4). The G-T effect leads to a prediction that step advancement occurs only when the length of the step exceeds a critical value, L_c , given by:

$$L_{c\pm} = 2\omega\langle\gamma\rangle_{\pm}/kT\sigma$$

where ω is area per growth unit; k , Boltzmann constant; T , temperature; and γ , the step edge free energy. Teng et al. (1998) also obtained the first estimates of the calcite step edge free energies, γ , and free energy barriers to one-dimension nucleation. (Note that γ (erg/m) is a fundamental parameter and is nonequivalent to the widely used but largely empirical bulk surface energy

(mJ/m².) With these relations, we were able to show that the introduction of aspartic acid, a common component in biomineralizing systems, dramatically affected growth morphology by decreasing the magnitude of the step edge energy. We subsequently found that the kinetics of step advancement were not affected by the addition of aspartate (Not presented in Teng et al., *Science*, 1998.) These findings indicate that aspartate modifies growth through changes in the thermodynamic state of the surface.

3. Surface Site-Specific Interactions of Aspartate with Calcite in Dissolution: Implications for Biomineralization (Teng and Dove, 1997, *Am. Min.*).

This study showed how organics can induce rapid morphological changes and suggested a complex role of organic compounds. Matrix macromolecules involved in regulating the biological growth of calcite are known to share a carboxylic-rich character. This arises from the abundance of acidic amino acids, aspartate (Asp) and glutamate (Glu). In this study we investigated 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 rhombic form. The introduction of Asp modified surface site reactivities to yield isosceles triangular etch pits (Fig. 5). With continued exposure to Asp-bearing solutions, 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 $(01\bar{1}1)$. Structural and stereochemical constraints suggest that these faces in the hillock are a combination of corresponding facets from the $\{1\bar{1}02\}$ and $\{1\bar{1}00\}$ crystallographic forms.

4. Kinetics of calcite growth: Surface processes and relationships to macroscopic rate laws (Teng, Dove, DeYoreo, 2000, GCA). Classical crystal growth theory is linked with observations of microscopic surface processes to quantify the dependence of calcite growth on supersaturation, σ , and show relationships to the same dependencies often approximated by affinity based expressions. *In situ* AFM was used to quantify calcite growth rates and observe transitions in growth processes on $(10\bar{1}4)$ faces in characterized solutions with variable σ . When $\sigma < 0.8$, growth occurs by step flow at surface defects, including screw dislocations. As σ exceeds 0.8, two-dimensional surface nucleation becomes increasingly important (Fig 6). The single-sourced, single spirals produced at lower σ were examined to measure rates of step flow and the slopes of growth hillocks. These data yielded the surface-normal growth rate, R , of (104) faces by the pure spiral growth mechanism.

The dependence of overall growth rate on dislocation source-structure was analyzed using the classical crystal growth theory. The resulting surface process-based rate expressions for spiral growth show the

relationships between R_m and the distribution and structures of dislocation sources. This is important because most dislocations exhibit complex structures with multiple sources

Figure 6

Figure 7

(Fig. 7). The analysis further shows how growth rate is highly dependent upon structure of the dislocation sources and growth mechanism (Fig. 8). The expressions resulting from this analysis were compared to affinity-based rate equations to show where popular affinity-based rate laws hold or break down.

Figure 8

Results of this study demonstrate that widely used second order chemical affinity-based rate laws are physically meaningful only under special conditions. The exponent in

affinity-based expressions is dependent upon the supersaturation range used to fit data. An apparent second order dependence is achieved only when solution supersaturations are very near equilibrium and growth occurs only by simple, single sourced dislocation spirals. These findings indicate the need for caution when deducing growth mechanisms and 'rate laws' from temporal changes in bulk solution chemistry.

5. Chirality dependent interaction between calcite and simple amino acids (Orme, Grantham, Teng, Noy, Dove, DeYoreo, accepted *Nature*). The effect of glycine, aspartic acid and glutamic acid on step speed, critical length and growth hillock, etchpits and crystal morphology was examined as a function of amino acid chirality and concentration. Addition of all amino acids during growth, led to stabilization of the (hk0) family of steps. When the amino acid had a chiral structure, the addition of pure L- and D- forms resulted in anisotropic expression of these steps so that the resulting growth hillock, etchpits and crystal morphologies displayed mirror symmetry about the glide plane of calcite as shown for aspartic acid in Figs. 9, 10 and 11. The addition of glycine, an achiral molecule, or D and L mixtures of aspartic and glutamic acid, led to symmetric forms as illustrated for glycine in Fig. 12. Because the overall facet shape exhibited the same shape as the growth hillocks, we were able to use the growth hillock geometry to construct the modified Wulff diagram for the orientational dependence of the step edge energy as shown in Fig. 13.

For any site on one side of the glide plane, an equivalent site on the other side can be found for which the geometric relationship with neighboring sites is related by mirror symmetry. Furthermore, the geometrical relationship between a single functional group of the amino acid with any single site on one side of the glide plane can be matched at the equivalent site on the other side. Consequently, the results of this study show unequivocally that the interaction of the amino acid with the calcite surface must involve both the chiral center of the amino acid (i.e., multiple groups) and multiple sites on the calcite surface. Through crystallographic modeling we showed that the differences between the geometrical relationship of L- and D-aspartic acids to the calcite {104}

Fig. 9 - Growth hillock morphology with D- and L-aspartic acid

Fig. 10 - Etch pit morphology with D- and L-aspartic acid

surface were subtly different, indicating that the energetic changes needed to drive the system from the D-induced to L-induced forms were small. This was consistent with the shallow character of the minima in the Wulff plot of Fig. 13.

In an attempt to experimentally determine the structural relationship between the adsorbed amino acid and the calcite surface, we recently performed *in situ* grazing incidence X-ray diffraction, NEXAFS and FTIR on calcite {104} faces grown in the presence of aspartic acid.

Despite the sub-monolayer resolution of these techniques, no evidence for an adsorbed layer was observed. Because the AFM data unequivocally show that such an interaction must exist, our conclusion is that the aspartic acid is adsorbed to the {hk0} faces, the same ones that are stabilized in the bulk crystals and that make up the step riser of the growth hillocks grown in the presence of aspartic acid. We are currently modeling the structure of an aspartic acid adsorption layer on the {110} and {1-10} faces for both the D and the L forms, and we are preparing samples for optical and X-ray spectroscopy of those faces in aspartic acid bearing solutions. In addition, before the current project is completed, we will have calculated the energy associated with ASP binding to these faces.

We showed that the addition of amino acids had little effect on the step speeds for the two types of steps on the calcite surface. In contrast, the terrace width (critical length) varied continuously with increasing amino acid concentration until, at high concentration, the well-defined step directions were lost as in Fig. 10. The results of this study demonstrate that the physical effect of the amino acids on the growth of calcite is to alter the surface energetics and not the step kinetics. In other words, modification of calcite by acidic amino acids is a thermodynamic effect, not a kinetic one.

IV. Summary

This project has been highly successful and we thank our sponsors for the opportunity to advance scientific knowledge in this rapidly advancing area of basic research.

Fig.13 -Wulff plot showing orientational dependence of step edge energy for pure calcite and calcite with L-ASP. Solid colored lines show real crystal shape. Dots show derived step edge energy. Solid black lines are schematic in regions where crystal shape gives no information.