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Biological Rules and Mechanisms Governing the Nanofabrication of Highly Regular Mineralized Microlaminate Composites

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We purified and characterized the proteins from the microlaminate abalone shell (a natural high-performance armor with fracture-toughness 3,000-fold greater than that of its mineral component alone) and "flat pearl" and from sponge and diatom biosilicas. We then cloned and sequenced the cDNAs encoding these proteins, and used the resulting structural information, in concert with site-directed mutagenesis (genetic engineering) and real-time atomic force microscopy and other advanced imaging techniques to reveal the fundamental mechanisms by which the proteins control the mineral nucleation, growth and nanocomposite structures based on both silicon and calcium. Advanced imaging with AFM, XRD, SICM, EDAXAX and NMR was used to resolve the mechanisms controlling synthesis and shape in biomineralization and biomimetic synthesis, and details of the structure-directing organic-inorganic interfacial interactions. We then used this information to make useful new mineral-organic composite materials in vitro.

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FINAL REPORT

Grant Number: N00014-93-1-0584

Principal Investigators: Daniel E. Morse, G. D. Stucky and P. K. Hansma

Institution: University of California at Santa Barbara

Grant Title: "Biological Rules and Mechanisms Governing the Nanofabrication of Highly Regular Mineralized Microlaminate Composites"

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OBJECTIVE: To identify and characterize the proteins, genes and molecular mechanisms governing the biological nanofabrication of mineralized composite materials, and harness these to develop new and environmentally benign routes to the synthesis of high-performance composites.

APPROACH: We purified and characterized the proteins from the microlaminate abalone shell (a natural high-performance armor with fracture-toughness 3,000-fold greater than that of its mineral component alone) and "flat pearl" and from sponge and diatom biosilicas. We then cloned and sequenced the cDNAs encoding these proteins, and used the resulting structural information, in concert with site-directed mutagenesis (genetic engineering) and real-time atomic force microscopy and other advanced imaging techniques to reveal the fundamental mechanisms by which the proteins control the mineral nucleation, growth and nanocomposite structures based on both silicon and calcium. Advanced imaging with AFM, XRD, SICM, EDAX and NMR was used to resolve the mechanisms controlling synthesis and shape in biomineralization and biomimetic synthesis, and details of the structure-directing organic-inorganic interfacial interactions. We then used this information to make useful new mineral-organic composite materials in vitro.

ACCOMPLISHMENTS: We discovered that the origin of both the exquisite nanostructural control and of the associated high-performance capabilities of these biomaterials lies in the unique mechanisms of their synthesis. In addition to the mechanisms of templating, epitaxy and polymer control of crystal nucleation and growth that were anticipated at the start of these studies, unanticipated new discoveries of self-assembling molecular stencils, novel self-healing energy-dispersive elastomers and structure-directing catalysts help explain the molecular mechanisms underlying the fabrication and performance of biologically produced mineral-organic composites. AFM analyses revealed that the microlaminate structure of nacre is organized over macroscopic dimensions by continuous growth of atomically coherent columns of protein-oriented aragonite through pores in a multilayered network of sheets of biopolymer that also are trilaminate. Stencil-like nanopores in these interlamellar matrix sheets guide the growth of the crystal columns from one layer to the next. The stochastic spacing of these nanopores apparently determines the lateral offset between successive microlaminae, thus generating the interdigitating brickwork that contributes to the material's remarkable strength. The sequence of Lustrin A cloned from this matrix reveals a strikingly repetitive,

alternating modular structure that helps explain its properties as an energy-dissipating elastomeric adhesive linker. Use of the atomic force microscope enabled us to measure the forces required to open successive domains in this modular elastomer, and to analyze its hysteretic "self-healing recovery. We thus discovered how the unique molecular structure and properties of Lustrin A contribute to the resiliency of the mineral-organic microlaminate composite. We discovered that silica needles are formed by a unique class of structure-directing, catalytically active proteins; we then used site-directed mutagenesis to identify the structural determinants of this catalysis.

These counterintuitive new findings, overturning the previous paradigms concerning the mechanisms of biological fabrication with both calcium and silicon, now are leading to the development of new low-temperature biomimetic routes to the synthesis of advanced mineral-organic composites. Use of the purified polyanionic proteins from the abalone shell allowed us to abruptly and sequentially switch crystallographic phase with stereospecific precision and fidelity, producing multiphase composites with micron-scale domains. We also used these proteins to produce crystalline thin-films of isostructural metal carbonates with useful optical, magnetic and electronic properties. Translating the molecular mechanisms of biosilica synthesis that we discovered to "biomimetic" chemical routes to new materials, we accomplished the first synthesis of mirrorless nanoscale lasers based on our biomimetically organized mesoporous silica channels.

CONCLUSIONS: Our principal conclusions are (1) that biology has evolved unique mechanisms for the synthesis and nanostructural organization of high-performance mineral-organic composite materials; (2) that these can be resolved through the use of biotechnology; and (3) that these mechanisms can then be harnessed to develop new low-temperature routes to high-performance magnetic, electronic and structural materials based on silicon and various metals.

SIGNIFICANCE:

This work represents the first purification, characterization and sequencing of proteins occluded within a biosilica, and the first discovery that such proteins and their cloned DNA can be used to chemically and spatially direct the polymerization of siloxanes in vitro. In addition to revealing the underlying molecular biological mechanism controlling the nanofabrication of silicon-based materials under mild physiological conditions, our findings demonstrate that this mechanism can be harnessed for the development of environmentally benign new routes to the synthesis of patterned silicon-based materials from a variety of tailored feedstocks.

Our studies of both the biosilica and calcium carbonate composites reveal the same fundamental lesson despite the vast differences in the chemistry and molecular biology of these two systems: In both, Nature uses two very different mechanisms to control mineral-composite synthesis and shape on the atomic scale and at the mesoscale level. Harnessing these mechanisms biomimetically, we have produced controlled silica architectures with control over porosity dimensions over length-scales ranging from angstroms to centimeters, with corresponding control of mechanical and optical properties yielding the first mirrorless nanoscale lasers. These, in turn, represent one of the first steps toward production of integrated optical circuits, analogous to the integrated electronic circuits that are in ubiquitous use today.

PATENTS:

- "Inorganic/Block Copolymer-Dye Composites and Dye-Doped Mesoporous Materials for Optical and Sensing Applications," Gernot Wirnsberger, Brian J. Scott, Nicholas A. Melosh, Howard C. Huang, Peidong Yang, Bradley F. Chmelka, Galen D. Stucky. 2000
- "Bifunctional Catalysts for Production of Silica, Glass, Silicones and Polymetallo-oxanes at Low Temperatures and Neutral pH." Daniel E. Morse, Galen Stucky and Yan Zhou. 1999.

AWARD INFORMATION:

- Co-PI Prof. Paul Hansma was honored by the American Physical Assocn. as the Outstanding Biological Physicist of the Year, 1999.
- Each of the 3 co-PIs was honored as Keynote Speaker at multiple International Symposia.

PUBLICATIONS:

- Fritz, M., A.M. Belcher, M. Radmacher, D.A. Walters, P.K. Hansma, G.D. Stucky, D.E. Morse and S. Mann. 1994. Flat pearls from biofabrication of organized composites on inorganic substrates. Nature 371: 49-51.
- Manne, S., C.M. Zaremba, R. Giles, L. Huggins, D.A. Walters, A. Belcher, D.E. Morse, G.D. Stucky, J.M. Didymus, S. Mann, and P.K. Hansma. 1994. Atomic force microscopy of the nacreous layer in mollusc shells. Proc. Royal Soc. London, B 256: 17-23.
- Giles, R., S. Manne, C.M. Zaremba, A. Belcher, S. Mann, D.E. Morse, G. Stucky and P.K. Hansma. 1994. Imaging single nacreous tablets with the atomic force microscope. Matrls. Res. Soc. Symp. Proc. 332: 413-422.
- Giles, R., S. Manne, S. Mann, D.E. Morse, G.D. Stucky and P.K. Hansma. 1995. Inorganic overgrowth of aragonite on molluscan nacre examined by atomic force microscopy. Biological Bulletin. 188: 8-15.
- Zaremba, C.M., A.M. Belcher, M. Fritz, Y.L. Li, S. Mann, P. K. Hansma, D.E. Morse, J.S. Speck and G.D. Stucky. 1996. Critical transitions in the biofabrication of abalone shells and flat pearls. Chem. Matrls. 8: 679-690.
- Belcher, A.M., X.C. Wu, R.J. Christensen, P.K. Hansma, G.D. Stucky and D.E. Morse. 1996. Control of crystal phase switching and orientation by soluble mollusc-shell proteins. Nature 381: 56-58.
- Proksh, R., R. Lai, P. K. Hansma, D.E. Morse and G.D. Stucky. 1996. Imaging the internal and external pore structure of membranes in fluid: TappingMode scanning ion conductance microscopy. Biophysical Journal 71: 2155-2157.
- Walters, D. A., B. L. Smith, A. M. Belcher, G. T. Palocz, G. D. Stucky, D. E. Morse, and P. K. Hansma. 1997. Modification of calcite crystal growth by abalone shell proteins: An atomic force microscope study. Biophysical Journal. 72:1425-33.
- Schaeffer, T.E., C. Ionescu-Zanetti, R. Proksch, M. Fritz, D.A. Walters, N. Almqvist, C.M. Zaremba, A.M. Belcher, B.L. Smith, G.D. Stucky, D.E. Morse and P. K. Hansma. 1997. Does abalone nacre form by heteroepitaxial nucleation or by growth through mineral bridges? Chem. Mater. 9: 1731-1740.
- Shen, X., A. M. Belcher, P.K. Hansma, G. D. Stucky, and D. E. Morse. 1997. Molecular cloning and characterization of Lustrin A, a matrix protein from shell and pearl nacre of *Haliotis rufescens*. J. Biol. Chem. 272: 32472-32481.
- Manne, S., T.E. Schaeffer, Q. Huo, P.K. Hansma, D.E. Morse, G.D. Stucky and I.A. Aksay. 1997. Gemini surfactants at solid-liquid

- interfaces: Control of interfacial aggregate geometry. Langmuir 13: 6382-6387.
- Belcher, A. M., P. K. Hansma, G. D. Stucky and D. E. Morse. 1998. First steps in harnessing the potential of biomineralization as a route to new high-performance composite materials. Acta Mater. 46: 733-736.
- Shimizu, K., J. Cha, G.D. Stucky and D.E. Morse. 1998. Silicatein α : Cathepsin L-like protein in sponge biosilica. Proc. Natl. Acad. Sci. 95: 6234-6238.
- Zaremba, C.M., D.E. Morse, S. Mann, P.K. Hansma, and G.D. Stucky. 1998. Aragonite-hydroxyapatite conversion in gastropod (abalone) nacre. Chem. Mater. 10: 3813-3824.
- Fritz, M. and D.E. Morse. 1998. The formation of highly organized biogenic polymer/ceramic composite materials: The high-performance microlaminate of molluscan nacre. Curr. Opin. Colloid Interface Sci. 3: 55-62.
- Cha, J.N., K. Shimizu, Y. Zhou, S.C. Christiansen, B.F. Chmelka, G.D. Stucky and D.E. Morse. 1999. Silicatein filaments and subunits from a marine sponge direct the polymerization of silica and silicones in vitro. Proc. Natl. Acad. Sci. USA 96: 361-365.
- Zhou, Y., K. Shimizu, J. N. Cha, G. D. Stucky and D.E. Morse. 1999. Efficient catalysis of polysiloxane synthesis by silicatein α requires specific hydroxy and imidazole functionalities. Angewandte Chemie Intl. Ed. 38: 779-782.
- Morse, D.E. 1999. Silicon biotechnology: Harnessing biological silica production to make new materials. Trends in Biotechnology 17: 230-232.
- Smith, B.L., T.E. Schäffer, M. Viani, J.B. Thompson, N. A. Frederick, J. Kindt, A. Belcher, G.D. Stucky, D.E. Morse and P.K. Hansma. 1999. Molecular mechanistic origin of the toughness of natural adhesives, fibers and composites. Nature 399: 761-763.
- Almqvist, N., N.H. Thomson, B.L. Smith, G.D. Stucky, D.E. Morse, and P.K. Hansma. 1999. Methods for fabricating and characterizing a new generation of biomimetic materials. Mater. Sci. Eng. C 7: 34-43.
- Morse, D.E. 1999. Silicon biotechnology: Proteins, genes and molecular mechanisms controlling biosilica nanofabrication offer new routes to polysiloxane synthesis. In: "Organosilicon Chemistry IV: from Molecules to Materials" (N. Auner and J. Weis, eds.); Wiley-VCH, New York; pp. 5-16.
- N.A. Melosh, P. Lipic, F.S. Bates, F. Wudl, G.D. Stucky, G.H. Fredrickson, and B.F. Chmelka, "Molecular and Mesoscopic Structures of Transparent Block Copolymer-Silica Monoliths," Macromolecules, 32, 4332-4342 (1999).
- F. Marlow, M.D. McGehee, D. Zhao, B.F. Chmelka, and G.D. Stucky, "Doped Mesoporous Silica Fibers: A New Laser Material," Advanced Materials, 11, 632-636 (1999).
- Cha, J. N., G. D. Stucky, D E. Morse, T. J. Deming. 2000. Biomimetic synthesis of ordered silica structures by block copolypeptides. Nature 403: 289-292.
- Stevens, R.M.D., N. A. Frederick, B.L. Smith, D.A. Walters, D.E. Morse, G.D. Stucky and P.K. Hansma. 2000. Carbon nanotubes as probes for atomic force microscopy. Nanotechnology 11: 1-5.
- P. Yang, G. Wirnsberger, S.R. Cordero, H.C. Huang, B. Scott, M.D. McGehee, G.M. Whitesides, B.F. Chmelka, S.K. Buratto, and G.D. Stucky, "Mirrorless Lasing from Mesostuctured Waveguides Patterned by Soft Lithography," Science, 287, 465 (2000).