

SPECTROMICROSCOPY AT THE ORGANIC-INORGANIC INTERFACE IN BIOMINERALS

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ABSTRACT. After a brief introduction to biomineralization, we describe x-ray absorption near-edge structure (XANES) spectromicroscopy, and how this approach explores the poorly understood mechanisms of biomineral formation. In particular, XANES identifies the molecular groups forming chemical bonds at the organic-inorganic interface. The first two experiments, on microbial polysaccharide-FeOOH mineralized filaments and on a synthetic peptide, are discussed.

INTRODUCTION

The Organic-Inorganic Interface in Biominerals

Biomineralization is the process by which organisms form minerals. Many biominerals are composite materials, containing a biologically produced organic matrix and nano- or micro-scale amorphous or crystalline minerals. Biomineral composite materials include bone, dentine, enamel, statoliths in the human ear, mollusk and crustacean shells, eggshells, algal and coccolith silica scales, radiolarian and diatom silica microskeletons and shells, and a variety of transition metal minerals produced by different bacteria (Banfield and Nealson, 1997; Fortin and others, 1997; Addadi and Weiner, 1997; Fitts and others, 1999; Templeton and others, 1999; Mann, 2001; Lower and others, 2001; Glasauer and others, 2002; Weiner and Dove, 2003; De Yoreo and Vekilov, 2003; De Yoreo and Dove, 2004).

Although the mechanisms of biomineral formation are not yet fully understood (for example, Mount and others, 2004), it is hoped that they may provide models for the fabrication of new materials and give new insights into the genetic control of biological structure (for example, Schäffer and others, 1997).

Biominerals provide a variety of functions to eukaryotes: protection, movement, grinding, and gravity or magnetic field sensing. Conversely, in prokaryotes, biominerals are often formed as a byproduct of a biochemical pathway, in which the bacteria oxidize or reduce transition metals or other inorganic solution species during metabolic energy generation (Banfield and Nealson, 1997).

In both cases, the complex bioinorganic chemistry involved in biomineralization constitutes a distinct evolutionary advantage for the organism performing it, which is why it became as widely spread as we observe today.

In the examples of eukaryotic biominerals given above, the biomineral products are clearly of direct benefit. In the case of microbial biomineralization, metal oxidation or reduction can be induced - or exploited - by the bacterium, but mineralization itself may have only indirect advantages or even disadvantages. Biomineral formation often occurs extracellularly, and it is subsequent to oxidation or reduction. In some cases it is detrimental: entombment of the bacterium in its own biomineral products is possible, and the cell either dies or develops an evasion strategy, such as the formation of mineral sheaths (for example, leptothrix) or stalks (gallionella) (Banfield and others, 2000).

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Many biomineralization mechanisms are poorly understood at the molecular level. These include all cases in which highly oriented crystals are formed with control over the particular crystal phase (polymorph) that is grown (De Yoreo and Dove, 2004). Shell, bone and some bacterial filaments are examples of such biomineralization: a highly specialized organic matrix directs the formation of a specific crystal phase and orientation. In these composites the organic-inorganic interaction is so specialized that a *templation mechanism* can be invoked. The particular matrix of organic molecules, produced by the living organism, acts as a template upon which crystals grow epitaxially, that is, growth is nucleated, and crystal structure, phase, orientation and often habit of the mineral are determined by the organic matrix.

It is conceivable that at the molecular scale, the organic molecule binds a few ions, which serve as nucleation site for crystal growth. Self-assembly and epitaxial crystal growth subsequently complete the composite structure, by filling the voids in the three dimensional organic matrix. Exploitation of such templation mechanisms, therefore, can be considered as a "genome shortcut", naturally selected to minimize the amount of information that the organism needs to transfer down the lineage, while maximizing the performance of the final composite material. Specifically, self-assembly and epitaxial crystal growth are harnessed by the organism, therefore the only necessary genetic information involves the production of the organic matrix.

From a materials science point of view, organic molecules are usually soft, but compliant and fracture resistant. Inorganic crystals are hard, but usually brittle. Biomineral composites exploit the best of these properties, and minimize the weaknesses: they are hard and fracture resistant. Nacre, for instance, is 3000 times tougher than geological aragonite! (Currey, 1977; Jackson and others, 1988; Schäffer and others, 1997).

Materials scientists have only recently begun to learn how to build a synthetic composite material that outperforms each component taken separately, and have done so inspired by shell nacre (Tang and others, 2003).

Nacre, or mother-of-pearl, of mollusk shells and pearls is a microlaminate composite of highly oriented calcium carbonate (CaCO₃) crystals and proteins (fig. 1), which exhibits exceptional regularity and mechanical strength (Mann, 2001). Nacre is an organic-inorganic composite, containing 500 nm thick aragonite (orthorhombic CaCO₃) crystal plates interspersed as "bricks" between organic matrix sheets, or "mortar" (Currey, 1977; Jackson and others, 1988; Schäffer and others, 1997). The organic matrix is composed of silk-like proteins and glycoproteins. These are, therefore, expected to be the templating molecules.

One interesting hypothesis explored by many authors is that the negatively charged amino acids along the protein sequences, aspartate and glutamate, attract positive ions from solution, and initiate crystal nucleation and growth (Mann, 2001; Weiner and Dove, 2003). This hypothesis deserves further investigation. We hereafter present preliminary data obtained from a synthetic polypeptide rich in glutamate, that binds Ca from solution.

A novel set of tools is necessary to discover exactly which molecules interact at the organic-inorganic interface, and at which specific molecular sites the first chemical bonds are formed, that is, how biomineral formation begins. X-ray spectromicroscopy, used in combination with other microscopic and biological methods, is one such novel tool to begin to unravel the chemistry of templation mechanisms.

Spectromicroscopy

Until recently, templation mechanisms have not been studied with x-ray absorption near edge structure (XANES) spectroscopy. The understanding of organicinorganic templates can be expanded by using XANES, because this powerful chemical



Fig. 1. Photograph of the nacreous inside face of an abalone shell (Haliotis rufescens). The shell width is approximately 20 cm.

analysis is sensitive to elemental composition, oxidation state, coordination number, and crystal or molecular structure of minerals and organic molecules (Stöhr, 1992).

XANES has been successfully used to reveal the presence and oxidation state of specific elements in geologic minerals (Sturchio and others, 1998), the structure of synthetic materials (Bozek and others, 1990), elemental speciation in soils and sediments (Myneni and others, 1997; Beauchemin and others, 2003; Zawislanski and others, 2003) and other environmentally relevant samples (Myneni and others, 1999; Myneni, 2002a).

Many other experiments on the microlocalization of trace elements in eukaryotic cells (De Stasio and others, 1993, 1996; Gilbert and others, 2000; De Stasio and others 2001) and the identification of prokaryotic biomineral products (Labrenz and others, 2000; Lopez-Garcia and others, 2003; Lawrence and others, 2003; Chan, De Stasio and others, 2004), also attest to the power and breadth of XANES spectroscopy and spectromicroscopy.

The lineshapes of XANES spectra correspond to the molecular and/or crystal structures surrounding the elements under analysis. However, interpretation of the spectral lineshape, and peak assignment can be complicated.

When the molecular or crystal structure is known, and relatively simple, *ab initio* calculations are used to simulate the XANES spectrum. A comparison of experimental and calculated spectra enables peak assignment to specific molecular structures. Specific peaks can be considered "spectral signatures" of specific molecular features. XANES is extremely sensitive to carbon chemistry: examples of molecular features generating well established spectral signatures are $C \equiv C$, C = C, C-O, C = O, C-O, as well as C-C-C bond angles, conjugation of adjacent bonds, *et cetra*. A material that contains several of these molecular features exhibits a XANES spectrum resulting from



Fig. 2. Top: Fe L-edge XANES spectra of the FeOOH mineralized looped filament shown in figure 5B, produced by an iron-oxidizing microbe. This spectrum is compared with iron oxyhydroxide standards, arranged (top to bottom) in order of increasing crystallinity, as measured by x-ray diffraction peak narrowing. Crystalline phases have well resolved peaks at ~708.4 and ~709.8 eV. As crystallinity increases the dip separating peaks (arrow) increases in depth. In the mineralized filament the lack of dip can therefore be interpreted as amorphous iron oxyhydroxide. Data from Chan, De Stasio and others, 2004.

the combination of the corresponding spectral signatures: the "building blocks" (Stöhr, 1992).

For other edges, for example, Si or S at the L-edge, simulations of XANES spectra are not currently adequate because the electronic structure is too complex to be calculated. In these cases, the spectral signatures do exist, and are measurable, but they are not univocally assigned to specific bonds or molecular structures. However, unknown minerals, such as sub-micron silicate inclusions, can still be identified by empirical comparison with spectra from known, macroscopic, reference silicate minerals (De Stasio and others, 2003; Gilbert and others, 2003).

XANES is also sensitive to crystallinity. In a recent experiment we observed for the first time a spectral signature at the Fe L-edge that measures the degree of amorphous versus crystalline mineral present in Fe^{3+} minerals (Chan, De Stasio and others, 2004). This signature is shown in figure 2.

Most importantly, XANES has been used to study the same kind of molecular interactions discussed hereafter, but without spatial resolution. For example, organic-inorganic interaction at the binding sites in metalloproteins (Benfatto and others, 2003), or between metal ion and humic macromolecules (Myneni, 2002a).

There are practical reasons that have completely precluded spectromicroscopy of biomineralized structure until now.

This spectroscopy can be performed by detecting fluorescence photons or photoemission electron (photoelectrons) from a solid sample surface. Fluorescence XANES signal is most intense at hard-x-ray energies, above 1 keV. However, the relevant absorption edges of organic elements N, C and O are: N K-edge 400 eV, C K-edge at 285 eV, and O K-edge at 531 eV. Since none of these edges is accessible to the x-ray fluorescence range, the organic components of biominerals have never been studied with fluorescence XANES.

Photoelectron XANES, also known as total electron yield or TEY-XANES, is much more intense than fluorescence below 1 keV, where the Ca L-edge, and the C, N and O K-edges are located. In this spectral region, a strongly space-averaged TEY spectroscopy has always been possible, on an insulating biomineral. This is, however, not particularly informative, given the highly organized microscopic structure of biominerals.

Spectromicroscopy with X-ray PhotoElectron Emission Spectromicroscope (X-PEEM) adds spatial resolution to the TEY experiment, down to the 10 nm level (Frazer and others, 2004). Until recently, however, X-PEEM could only image and analyze the chemistry of conductive sample surfaces. Insulating samples such as minerals and biominerals could not be analyzed without major charging problems.

Transmission x-ray microscopy experiments (for example, scanning transmission x-ray microscopy, STXM (Kilcoyne and others, 2003; Tyliszczak and others, 2004), which do not suffer from charging, are limited to very thin solid samples (few atomic monolayers) or dilute liquid samples. Biominerals, therefore, are excluded from this powerful analysis.

We recently optimized a differential-thickness coating method (De Stasio and others, 2003) that enabled us to extensively study mineral surfaces with X-PEEM and do high-resolution imaging and XANES analysis on them (35 nm or better) (Gilbert and others, 2003). We have used this coating approach on a variety of insulators, including wood, quartz, zircons, glass slides, tribological polyphosphate films and cells. In all these cases the coating completely removed charging and enabled micro- and nano-XANES spectroscopy of insulators. Figure 3 shows a representative example of the results enabled by differential-thickness coating.

X-PEEM, combining XANES spectroscopy and spatial resolution, can study the molecular interactions involved in biomineral formation. It can, potentially, analyze the organic and inorganic components of biominerals *in situ*. It is, therefore, the most promising technique to investigate biominerals, and understand the interactions of the organic matrix with the minerals, through their spectral signatures.

Once carbon XANES spectra from the bound, mineral-templating, and unbound organic matrices of biominerals are obtained, the difference between those spectra reveals the organic-inorganic interaction. Interpretation of the data is then done by comparison with the extensive literature on carbon XANES spectroscopy in individual amino acids and organic compounds (Stöhr, 1992; Carravetta and others, 1998; Kaznacheyev and others, 2002; Myneni, 2002b), or by comparison with reference molecules prepared ad hoc for a specific interaction.

Two experiments described below will clarify these methods.

The major weakness of the XANES X-PEEM approach is the inability to separate co-localized mixed phases. In the presence of multiple proteins in a biomineral (for example, bone), carbon K-edge spectra may be too complicated to interpret. In that case it is necessary to acquire spectra from separate single-components, and deconvolute individual contributions to XANES spectra of the mixture. Separation and/or purification of single components may not be possible. Furthermore, the components may not be spectroscopically distinguishable. If the individual organic components



Fig. 3. Distribution maps of quartz (magenta) and zircon (blue), extracted from XANES stacks of images (each pixel contains the full Si L-edge XANES spectrum). These data were acquired using the SPHINX¹ instrument, which is an X-PEEM (Frazer and others, 2004), on the polished surface of the oldest zircon known - 4.4 billion year old (Wilde and others, 2001) - and superimposed on the direct image of the same zircon (cyan). The quartz map shows quartz inclusions in the zircon. The smaller inclusion is 800 nm in diameter: too small to be analyzed with any other non-destructive technique. (Data from De Stasio and others, 2003).

contributing to XANES spectra are known and spectroscopically distinct, singular value decomposition, or cluster analysis methods can be used to deconvolute and quantify their contributions (Pickering and others, 2000; Lerotic and others, 2004).

RESULTS AND DISCUSSION

First Experimental Observation of an Organic-Inorganic Template: Bacterial Polysaccharides and Akaganeite

We recently analyzed a mineral structure in which an organic-inorganic template is the only possible explanation for the formation of the crystal observed (Chan, De Stasio and others, 2004).

Iron-oxidizing bacteria, that have not yet been isolated nor phylogenetically identified, induce the formation of unprecedented ~ 2 nm wide, up to 10 micronlong, curved pseudo-single crystals of akaganeite (b-FeOOH) in the cores of filaments. The filaments are 20 to 200 nm wide, tangled, and composed of 2-line ferrihydrite (FeOOH \cdot nH₂O), surrounding the akaganeite cores (fig. 4). Formation of akaganeite in solution requires the presence of chloride, and is unexpected in fresh water. We therefore suggested that the mechanism of akaganeite formation requires templation by organic polymers extruded by the bacteria (Chan, De Stasio and others, 2004).

A key step in the formation of the akaganeite filament cores is the formation of chemical bonds between an organic molecule and solution or precipitated species, precursors to the inorganic crystal. As in many other biominerals, the organic molecule acts as a template for a particular mineral polymorph.

We first analyzed the polymer fibrils extruded from the bacterial cell membrane, using the SPHINX X-PEEM (Frazer and others, 2004), and identified them as

¹Spectromicroscope for PHotoelectron Imaging of Nanostructures with X-rays (SPHINX), installed on the High Energy Resolution MONochromator (HERMON) beamline, at the Wisconsin-Synchrotron Radiation Center.



Fig. 4. SEM image of mineralized filaments produced by Fe-oxidizing bacteria in the Piquette abandoned and flooded mine in Tennyson, WI. The filaments, approximately 100 nm in diameter are mineralized by FeOOH adhesion to the polysaccharide chains immediately after being extruded by the bacterium. On the right hand-side of the image a thinner, faint, strand is visible, possibly a non-mineralized polysaccharide fibril. Scale bar is 1 µm. (Imaged by B. H. Frazer and G. De Stasio)

polysaccharides by comparison of their carbon K-edge XANES spectra, with those from representative reference compounds (fig. 5).

We then analyzed the mineralized filaments from the biofilm. These also revealed a polysaccharide spectrum at the carbon K-edge. FeOOH nanoparticles formed an ~ 50 nm thick coating around the polysaccharide fibrils, hence the carbon signal was much lower, relative to the ~ 2 nm diameter uncoated filaments.



Fig. 5. SPHINX images and spectra of the filaments produced by iron-oxidizing bacteria. (A) Image of non-mineralized polymer fibrils from the cloudy water above the biofilm (5 μ m scale bar) and (B) mineralized filaments from the biofilm (1 μ m scale bar). These, and all other mineralized filaments in the biofilm, contain the akaganeite crystal core described in the text. (C) Carbon K-edge XANES spectra from multiple non-mineralized (NM) fibrils in (A), the single, looped, 100 nm wide, mineralized (M) filament in (B), and reference organic molecules: sodium alginate (acidic polysaccharide); albumin (protein); a single chain C-16 saturated lipid; and calf thymus DNA. The dashed lines at 287.3 eV and 288.6 eV highlight the energy position of the most characteristic peaks for DNA and alginate. Notice the similarity of the spectra from the NM fibrils and M filaments with the polysaccharide spectrum, and the additional structure in the one from the M filament: the peak at 292.4 eV was assigned to the C-O bond in carboxyl groups. Data from Chan, De Stasio and others, 2004.

With regard to biomineralization, the most relevant result of that experiment is that carbon spectroscopy from mineralized filaments revealed a new peak, which was never present in spectra from the non-mineralized polysaccharide strands. This spectral signature was interpreted as a s* resonance of a C-O single bond involved in FeOOH binding. It is likely that the C-O groups that interact with FeOOH originate from the carboxyl groups (O = C-O⁻) of acidic polysaccharides (for example, alginate). Acidic polysaccharides have an excess of COO⁻ groups that have high affinity for binding positive ions.

We concluded that carboxyl groups in the unidentified biofilm polysaccharide chains must be sites at which FeOOH amorphous nano-precipitates bind, initiating templation and akaganeite crystal formation upon ageing (fig. 5).

Biomineralization of these bacterial filaments has common features with mollusk shell nacre formation: in both cases there is enough evidence to suggest that negatively charged groups along the organic polymers are responsible for the interaction with the positively charged mineral ions, Fe^{3+} and Ca^{2+} , respectively. In the case of the microbial acidic polysaccharides, negatively charged COO⁻ groups are responsible. Along the acidic glycoproteins in nacre it is believed that the negatively charged amino acids glutamate and aspartate, play an equivalent role (Mann, 1988, 2001; Mann and others, 2000; Weiss and others, 2000; Weiner and Dove, 2003).

Acidic amino acids or polysaccharides with excess carboxyl groups are the most common and most effective cation-binding biopolymers that any organism can assemble to bind mineral precursors and initiate templation.

Analyzing a biomineral from both the mineral and the biopolymer perspective, it is always possible to identify spectral signatures even if the bond sites are not those expected. As shown by the recent experiment on microbial polysaccharide–FeOOH filaments, by XANES analysis of the organic and the mineral components of biominerals, the nature of the organic-inorganic interactions is determined, thereby revealing the beginning of templation at the molecular scale.

XANES Analysis of a Designed Peptide

The major complication in understanding the formation and templation of biominerals lies in the discovery of multiple proteins in each biomineral. Several of these proteins in combination may play a role in biomineralization. Some exhibit specific binding sites (for example, aspartate and/or glutamate), others have catalytic (Weaver and Morse, 2003) or a stereochemical recognition function (De Yoreo and Dove, 2004).

A class of relatively simple proteins, naturally optimized to bind crystals is antifreeze proteins (AFPs). AFPs provide fish and grass with protection against the freezing effects of polar environments by binding to ice surfaces and inhibiting growth of ice crystals (Madura and others, 1994; Sicheri and Yang, 1995; Zhang and Laursen, 1998). Ice is not generally considered to be a "biomineral", but the AFPs represent the best available structural models for interactions between a peptide and an inorganic surface. Detailed structural hypotheses have been proposed to explain how specific AFP surfaces (from x-ray crystallography or multi-dimensional NMR spectroscopy) bind to specific ice crystal faces. As far as we know, there are no comparable models for interactions between true biominerals and the proteins or other biopolymers that control their formation; indeed, there is no structural information for the relevant proteins by themselves. Therefore, extrapolation from known AFP structures, and current understanding of how AFPs interact with specific ice surfaces may lead to more immediate insights in biomineralization.

Several structural classes of AFPs have been identified; all have relatively simple folding patterns. The simplest are the type–I AFPs, which form a single long α -helix with ice-binding side chains (such as the hydroxyl-bearing side chain of threonine)

aligned along one side. High-resolution structural data for other AFPs suggest that β -sheet secondary structure can also provide a surface for display of an appropriately organized set of crystal-binding side chains (Leinala and others, 2002).

Evans has recently suggested that perhaps the proteins involved in antifreeze and biomineralization functions are not truly comparable, since the latter tend to be unfolded in the absence of the mineral (Evans, 2003). Recent work of Hamilton and others, however, shows that molecules designed to display arrays of carboxylate side chains designed to share a common orientation can influence the growth of CaCO₃ crystals, and that the influence can be rationalized on an atomic scale (Estroff and others, 2004). These results strongly support our approach: as a biomineral model, we analyze synthetic peptides inspired by AFPs, and the interaction of these with ions and nanoparticles in solution.

We designed a 20-residue peptide, **1**, to be capable of interaction with biomineral surfaces, based on principles manifested by type I AFPs. Peptide 1 is designed to adopt an α -helical conformation (favored by the numerous alanine (Ala) and glutamine (Gln) residues) that displays a "stripe" of six glutamate (<u>Glu</u>) residues along one side. Placing the Glu residues in a repeating pattern (i, i + 3, i + 7, i + 10, i + 14, ...), the so-called 3,4-repeat, leads to alignment of these residues along one side of the helix. At neutral pH or higher, the carboxyl side chains of the Glu residues should be ionized; thus, **1** should display a linear array of six anionic carboxylates, allowing it to interact with an array of Ca cations at the surface of a mineral.

Ac-Ala-<u>Glu</u>-Gln-Gln-<u>Glu</u>-Ala-Gln-Gln-<u>Glu</u>-Ala-Ala-<u>Glu</u>-Gln-Ala-Ala- · · · ·

\cdots <u>Glu</u>-Gln-Ala-<u>Glu</u>-Ala-NH₂ = Peptide 1

Peptide 1 was analyzed with XANES spectromicroscopy. The results show that binding of Ca^{2+} ions to the peptide exerts a significant effect on the chemical bonds within the peptide, as demonstrated by the spectral modifications observed at the carbon K-edge.

In figure 6 we present the C K-edge spectra for peptide 1 with and without Ca²⁺ ions in solution. The spectra show the effect of Ca-binding on the carboxyl group electronic structure. In particular, the C = O peak at 288.4 eV is shifted by 0.2 eV towards higher binding energy, but it is also broadened and its lineshape is altered when peptide 1 binds Ca. The interpretation of spectral signatures in XANES spectroscopy of large molecules (> 1 amino acid) is complex, and requires further interpretation. Fitting of the peaks in these two spectra is helpful. In figure 6 we show that the main spectral feature in peptide 1 is the π^* resonance of the C = O groups, and that these are best interpreted as superposition of 3 Gaussian peaks.

The peak fitting results show a dramatic difference between bound and unbound carboxyl groups in peptide 1. Specifically, upon Ca²⁺ binding, peak 2 is depleted, while peaks 1 and 3 are enhanced. Because carboxyl groups in glutamate are the most likely binding sites in peptide 1, and because the most striking differences in the spectra are at the C = O spectral signature, it is reasonable to conclude from these results that any peptide, protein, glycoprotein or polysaccharide with excess carboxyl groups can probably be studied by XANES spectroscopy and spectromicroscopy. In particular, the difference between bound and unbound organic molecules in the presence of nanoparticles can be observed with XANES spectroscopy. In addition, this difference can now be observed *in situ*, within solid, composite, natural biominerals.

Other studies on the sensitivity of XANES to protein structure and folding are currently being conducted. These might reveal that protein folding is altered by mineral binding, yielding yet another component for the interpretation of XANES spectra from biominerals.



Fig. 6. Carbon K-edge XANES spectra of peptide 1 (A) and peptide 1 + Ca (B). Both samples were dried droplets of 100 μ m solution of peptide 1, with and without 681 μ M CaCl₂. Note the shift and broadening of the Ca-bound peptide 1 spectrum. Peak fitting in both cases is optimized ($\chi^2 < 10^{-4}$) using 4 Gaussian peaks (solid black lines), and 1 arctangent (dotted line). The experimental spectra are reported with black dots and no line. The green line closely approximating the experimental data is the fit obtained with the above components. The red line is the residue, that is, the difference between the experimental data and the fit. The 4 Gaussian peaks are identified by corresponding numbers in the two plots. The first peak at 287.3 eV has minimal intensity in A (< 0.1 in normalized intensity arbitrary units), while it is prominent in B. Peak 2 is much more intense in A than in B (1.73 vs 1.24, and shifted from 288.1 to 288.2 eV), while peak 3 is less intense in A than in B (0.3 vs 0.6, and shifted from 288.8 to 289 eV). These 3 peaks are associated with the C = O π^* resonance, while peak 4, centered at 296.6 eV, corresponds to the broad σ^* resonances of C-C, mainly in the side chains of peptide 1.

CONCLUSIONS

There are many gaps in the present knowledge about biomineral formation and templation mechanisms. XANES studies of molecular structures in biominerals might reveal some of the microscopic details of this phenomenon. If one accepts the hypothesis that composite biominerals form as a result of complex chemical interactions between organic and inorganic matrices, and that the former acts as a template for the latter, then the interface between the two must be analyzed to elucidate templation mechanisms.

Our recent experiments on mineralized microbial filaments and on a synthetic peptide designed to bind Ca^{2+} represents the first evidence that the organic-inorganic interface can be understood with spectromicroscopy. We look forward to seeing the same nano-analytical approach used by other groups and ours, and extended to reveal the organic-inorganic interface in other biomineral systems.

Once the molecular-scale chemistry of the interface will be elucidated, it might be possible to harness it and synthesize novel biomimetic composite materials that self-assemble and outperform the sum of their components.

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