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Letter

Backbone Structure of Diatom Silaffin Peptide R5 in Biosilica Determined by Combining Solid-State NMR with Theoretical Sum-Frequency Generation Spectra

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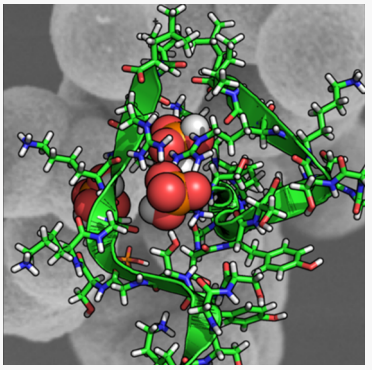
ABSTRACT: Silaffin peptide R5 is key for the biogenesis of silica cell walls of diatoms. Biosilification by the R5 peptide has potential in biotechnology, drug development, and materials science due to its ability to precipitate stable, high fidelity silica sheets and particles. A true barrier for the design of novel peptide-based architectures for wider applications has been the limited understanding of the interfacial structure of R5 when precipitating silica nanoparticles. While R5−silica interactions have been studied in detail at flat surfaces, the structure within nanophase particles is still being debated. We herein elucidate the conformation of R5 in its active form within silica particles by combining interface-specific vibrational spectroscopy data with solid-state NMR torsion angles using theoretical spectra. Our calculations show that R5 is structured and undergoes a conformational transition from a strand-type motif in solution to a more curved, contracted structure when interacting with silica precursors.



The biomineralization of bone, teeth, tusks, protective shells, and other hard tissue has been the envy of material scientists for decades. There are a broad variety of potential applications for environmentally friendly, efficient, and biocompatible protein-mediated mineralization in fields such as regenerative medicine, catalysis, electronics, and optics.[1−4]

Silica precipitation by diatoms (*Bacillariophyceae*) has played an important role in this context.[2,5] High-fidelity nanostructured silica is potentially of interest for industrial applications. Biosilica, as one of the most studied biominerals, is an important model system for the molecular processes involved in mineral biogenesis. In vivo silica formation is driven by type-1 silaffin, a post-translationally modified phosphopeptide, and long-chain polyamines (LCPAs),[6] both of which become embedded in the silica precipitates in the process. The thus-obtained materials consist of spherical silica particles with diameters of about 500 nm. Kröger and coworkers have also shown that an unmodified peptide chain based on repeats within the silaffin-1A1 sequence, called R5 (SSKKSGSYSGSKGSKRRIL), can precipitate materials that resemble the structures formed in vivo.[2] A scanning electron microscopy image of a biosilica nanosphere formed by R5 is shown in Figure 1a.

Despite the central role R5 has been playing in the investigation of new biosilica nanostructures and biomineral studies in general,[2] there have only been a few structural studies of the formation of nanostructured silica by R5 at the molecular level. While direct structural studies of R5 within particles are extremely challenging, recently developed methods to probe proteins at flat surfaces can be more readily applied to biosilification.[7−9] A recent study of R5 silica precipitation at the air−water interface showed that at flat surfaces R5 assembles into peptide monolayers and, in the presence of silica precursors, the peptide can induce the formation of nanometer-thick silica sheets (Figure 1b).[10] A combination of vibrational sum-frequency generation (SFG) and molecular dynamics (MD) simulation showed that R5 binds the air−water interface via its RRIL motif (Figure 1c). The study revealed that the hydrophobic interaction of the C-terminal RRIL sequence with the air−water interface stabilizes the alignment and orientation of R5 and thereby exposes the lysine-rich N-terminal regions to the tetramethyl orthosilicate (TMOS) precursors in the solution.

Structural studies of R5 precipitating silica nanoparticles, on the other hand, have been sparse and contradictory. Knecht and Wright first proposed that R5 forms micelles in solution, which are stabilized by the C-terminal RRIL motif, reminiscent of the hydrophobic interactions at the air−water interface.[11] The molecular cartoon in Figure 1d is based on this work. Later, solid-state nuclear magnetic resonance (ssNMR) studies by Drobny et al. supported this view and suggested that R5 is structured in solution and that the silica interaction would take

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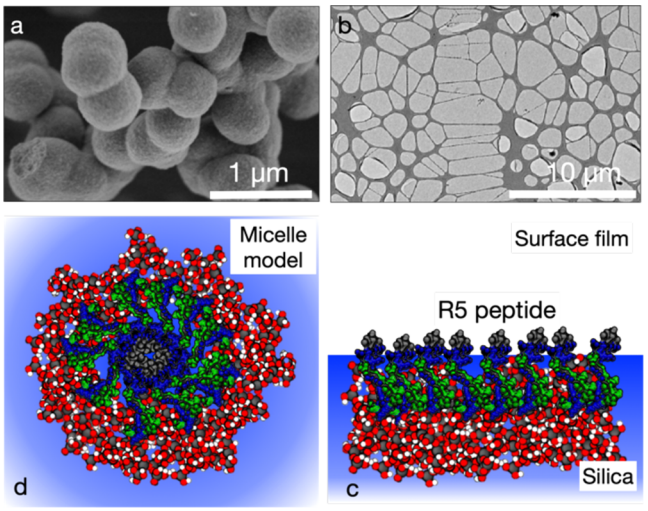

Figure 1. Biosilica precipitation by silaffin-derived R5 peptide at the surface and in bulk solution. (a) Biosilica nanospheres precipitated by R5 in solution (reproduced after ref 10). (b) TEM image of a biosilica sheet precipitated at the air–water interface on a Lacey grid. The dark lines show the grid, and the light areas are covered with silica sheets.[10] (c) At the air–water interface, R5 assembles via isoleucine–leucine sites (gray) and precipitates silica via lysine and arginine side chains (blue). (d) Model of R5 assembly and interaction with silica in solution. The micelle model has been proposed to explain the formation of particles shown in (a).

place predominantly via the N-terminal lysine residues, while the RRIL sites are buried within the hydrophobic core of the micellar structures.[12] The model of ordered R5 in solution and precipitation through micellar structures has been opposed by a solution-state NMR study by Senior et al.[13] Their analysis showed that R5 maintains an unstructured and monomeric state in solution and that silification takes place along the entire peptide sequence rather than exclusively via the N-terminal lysine sites. Besides the two NMR studies, self-assembly processes such as organic matrices and phase separation have also been proposed.[6]

The similarity of the surface assembly predicted by SFG and MD on flat surfaces and the molecular geometries within a possible micelle structure, both depicted in Figure 1, is striking. If peptide assembly and subsequent silica interactions at the highly curved micelle geometry are comparable to biosilification at a flat model surface, then the original precipitation model proposed by Knecht and Wright is most likely accurate. A significant deviation of the R5 structure in solution from the conformation found at interfaces would indicate that the micelle model is incorrect. The difficulty has been to reconcile the ssNMR structural data based on the micelle model with any surface-specific spectroscopic data. To make that connection and help solve the long-standing puzzle of bioprecipitation of silica nanospheres, here we combine previously published, bulk-sensitive ssNMR data with surface-specific SFG experiments using theoretical spectral calculations.

Vibrational SFG spectroscopy in the amide-I region can provide detailed information about the folding of peptides and proteins at interfaces.[14] It is therefore ideally suited to probing proteins involved in hard tissue biogenesis at material surfaces. One challenge has been the interpretation of SFG amide-I spectra of complex proteins. Since SFG is a coherent type of spectroscopy and signals generated at different sites along the peptide chain interfere in complex ways, it is often impossible to determine the secondary structure and orientation by spectral inspection or peak fitting.[14] This is especially true for peptides with nonstandard strand-like folding, such as those observed for mineralizing peptides.[15,16] One way to solve this problem is to calculate theoretical SFG spectra from protein structures and compare the calculated spectra with experimental spectra. The protein structures can, for example, be obtained from crystal structures stored in the protein data bank (PDB) or from MD simulations. This strategy has been successfully applied to artificial peptides,[17] membrane proteins,[18] amyloid peptides,[19] blood proteins,[20] and ice-binding proteins.[21]

In this study, for the first time, ssNMR data are used to model SFG spectra. NMR-derived dihedral backbone angles obtained from precipitated particles[12] are combined with recently reported SFG spectra recorded at an equivalent protein–silica interface.[10] Figure 2a shows the structure of R5 constructed from ssNMR dihedral angles for lyophilized R5 powder and after interaction with silica. The dihedral angles used in the model are given in Table S1.

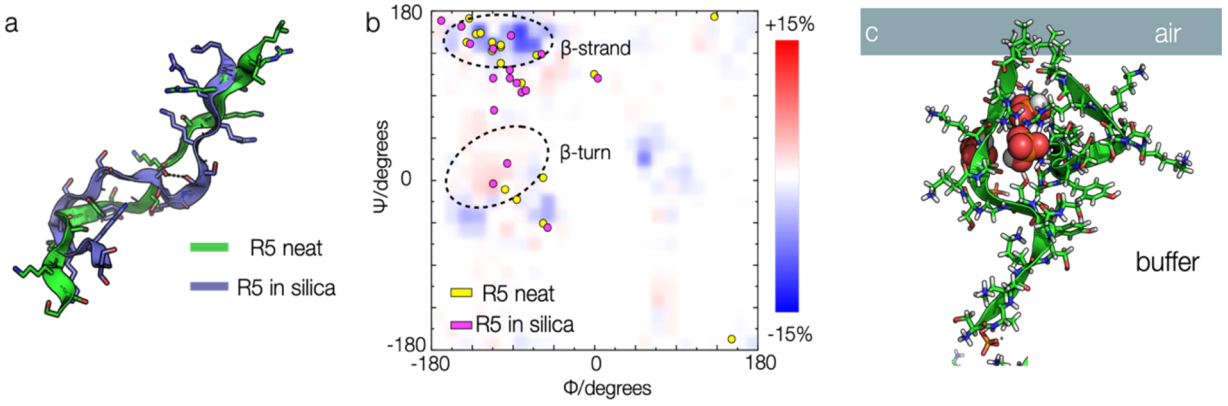

Figure 2. (a) R5 peptide in the dry state (green) and in biosilica (blue) based on ssNMR dihedral angles obtained for lyophylized peptides and silica nanoparticles. The silica model is shown with $\Phi = -119°$ and $\Psi = 113°$ (consistent with the angles of surrounding residues) for S7 and Y8 because the backbone chemical shifts did not give a robust assignment. (b) Ramachandran plot of ssNMR-derived backbone dihedral angles for neat R5 (yellow circles) and in biosilica (magenta circles), along with the difference Ramachandran plot calculated from MD simulations before and after TMOS interaction.[10] (c) Snapshot of MD simulation of R5 peptides interacting with TMOS at the air–water interface.

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By analogy to previous calculations based on PDB structures and MD trajectories, we have used these NMR R5 structures as the basis for SFG spectral calculations. In the previous SFG experiments, first the R5 peptides are injected into a Teflon trough and allowed to assemble at the water surface.[10] To avoid mineralization across the bulk solution, the subphase is exchanged by buffer solution three times to decrease the bulk peptide concentration. After the first set of spectra is recorded, tetramethyl orthosilicate (TMOS) is injected into the subphase. Following the precipitation of a nanometer-thick silica composite film at the air−water interface, a second set of spectra is recorded.[10] Figure 3a,b shows experimental and calculated SFG spectra of R5 at the air−water interface before and after silica precipitation recorded in ssp (s-polarized SFG, s-polarized vis, and p-polarized IR) and sps polarization. The experimental ssp spectra contain a relatively broad feature with a peak frequency that shifts from ∼1685 to 1670 cm^{-1} upon the addition of TMOS, which is typically assigned to β-sheet modes and turn structure, respectively.[22] The sps spectra (Figure 3b) show a resonance near 1650 cm^{-1}, which can be assigned to an α-helix or random coil, with the latter assignment being more likely here.

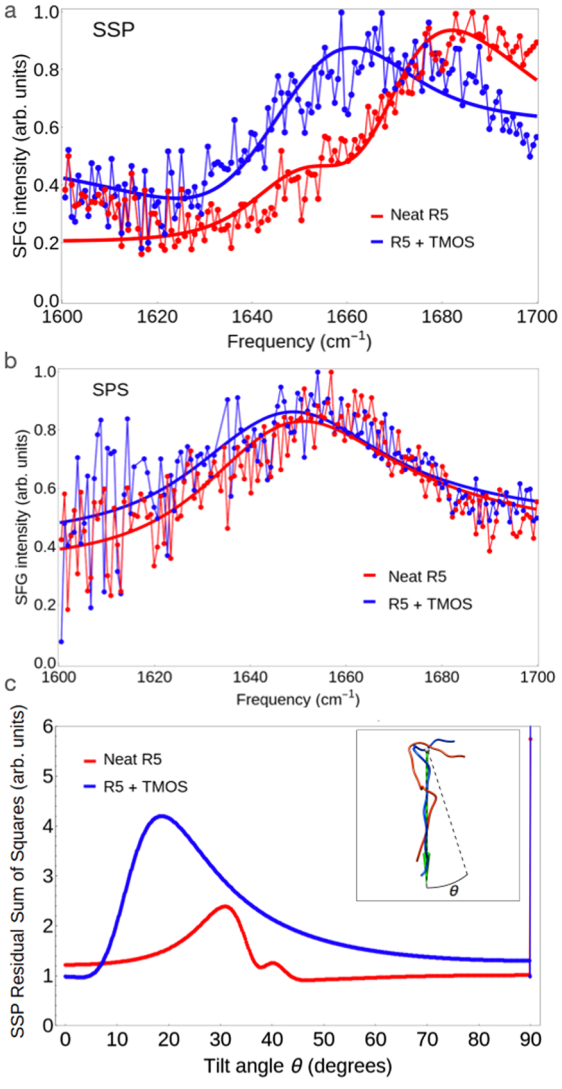

Figure 3. (a, b) Experimental (thin dotted lines) and calculated (thick lines) SFG spectra of R5 at the air−water interface before and after interaction with the TMOS silica precursor in ssp and sps polarization combinations, respectively. The calculated spectra are based on the ssNMR-derived dihedral angles and calculated for a tilt angle of 0°. (c) The residual sum of squares values. At angles >85°, the fits no longer converge.

We calculate theoretical spectra with a one-exciton Hamiltonian model based on the ssNMR-derived dihedral angles (more details in the SI) for a series of tilt angles. To keep the spectral model as simple as possible, we assume that there is uniaxial symmetry around the seven C-terminal residues of the peptide. Since ssp shows the most significant variance of the spectral match with peptide structure in this case, we focus our analysis on this polarization combination first. The ssp spectra for neat R5 match best for small θ values and angles larger than 60°, as expressed by the residual sum of squares (RSS) depicted in Figure 3c (the peptide tilt angle θ is defined in the inset in the same figure). The solid lines in Figure 3a,b show the close similarity between the experimental and calculated spectra for a $\theta = 0°$ orientation. On the basis of previously reported MD simulations at flat surfaces, a perpendicular orientation with respect to the surface (i.e., small θ) would be expected, which is in agreement with the calculation results.[10] At the same time it should be noted that, apart from intermediate angles around 20−30°, the RSS values do not vary strongly with the peptide orientation. Since the purpose of the calculations is to compare the R5 structure obtained from NMR and SFG, this is a reassuring result. It indicates that the predicted red shift in the calculated spectra is based on a changing peptide configuration upon TMOS interaction and is not the result of a difference in peptide orientation.

The red shift observed when going from neat to TMOS-interacting R5 indicates a loss of β-strand content, which agrees well with the ssNMR dihedral angles (Figure 2) and a transition from an extended, strand-like fold to a more contracted, coiled folding motif. The sps spectra, which do not exhibit a red shift upon TMOS interaction, also show excellent agreement between calculated and experimental spectra.

Given the difference in sample condition for ssNMR (dry peptide) and SFG (solution state), it is reassuring to note that the structural transition observed with both methods is also borne out in previously reported MD data.[10] Figure 2b shows the difference Ramachandran plot extracted from the MD simulations reported in ref 10 of R5 at flat surfaces before and after TMOS interaction. The plot shows a reduction of β-strand content and the appearance of additional β-turns.

The ssNMR torsion angles before and after TMOS interaction shown in the Ramachandran plot follow the same trend. An exemplary snapshot of the MD structures obtained at flat surfaces (Figure 2c) illustrates the R5 structure when interacting with silica. The peptide chain bends to follow the shape of the silica clusters, very similar to the in-silica ssNMR structure shown in Figure 2a. It is interesting that such a contraction of strand-like peptides has also been observed during the mineralization of calcium carbonate and calcium oxalate.[15,16] The biological function could be to maximize the contact of peptide lysine sites with intercalating silica clusters during precipitation.

Evidently, the R5 precipitation of composite nanospheres in solution and sheets at the air−water interface commences via very similar structural motifs. The excellent agreement of SFG and ssNMR spectral data shows that R5 is structured before silica interaction, which makes a micelle-type assembly a very

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likely scenario. The agreement between the ssNMR and SFG data, correlated via the spectral SFG calculations, indicates that R5 maintains a defined conformation within silica particles, by full analogy to the interface assembly. The hydrophobic terminus drives the assembly of the peptides, which in turn determines the morphology and shape of the obtained biosilica. The robustness of the working principles opens up a new avenue for the synthesis of biosilica structures. By tuning the hydrophobic interaction of an artificial peptide or variants of the R5 sequence and thus designing new hydrophobic contacts in solution, emulsions and, at artificial surfaces, a variety of biosilica structures could be achieved on the basis of the same design principles as used by nature. It has been shown that R5 is not limited to silica precipitation but can also precipitate titanium oxide and germanium oxide structures.[23,24] The versatility and robustness of R5 biosilication are therefore a promising route not only toward new biosilica architectures but also toward a variety of other functional nanostructured materials.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpclett.1c02786.

NMR methods, NMR sample preparation, SFG methods, SFG sample preparation, spectra calculations, and table of NMR torsion angles (PDF)

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Notes

The authors declare no competing financial interest.

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