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Letter

Structure of Keratins in Adhesive Gecko Setae Determined by Near-Edge X-ray Absorption Fine Structure Spectromicroscopy

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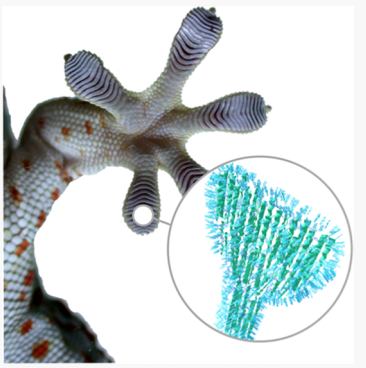
ABSTRACT: Geckos have the astonishing ability to climb on vertical surfaces due to the adhesive properties of fibrous setae at the tips of their toe pads. While the adhesion mechanism principle, based on van der Waals interactions of myriads of spatula located at the outermost end of the setal arrays, has been studied extensively, there are still open questions about the chemistry of gecko setae. The gecko adhesive system is based on keratin fibrils assembled to support the entire setal structure. At the same time, the structure and alignment of keratin molecules within the ultrafine spatula tissue, which can support the enormous mechanical strain, still remain unknown. We have studied the molecular structure of gecko spatula using near-edge X-ray absorption fine structure (NEXAFS) imaging. We indeed found that the setae consist of a β-sheet structure aligned with the adhesion direction of the setae. Such alignment may provide mechanical stability to the setae and resistance to wear across different length scales.



The gecko's amazing adhesive skills, which enable it to climb on nearly every surface in almost any condition, have provided inspiration for biomimetic applications in adhesion technologies.[1−4] The adhesive properties of the gecko toepad are based on the hair-like fine structure of fibrous setal arrays.[1,2,5] The seta split up into bundles of spatula shafts, each tipped with flat spatula at the distal end, which make the final contact to the surface.[5−8] The adhesive system of geckos has been studied extensively. Autumn et al.[2,3] discovered the interaction of gecko spatula with surfaces is mainly driven by van der Waals interactions. At the same time, the molecular geometry of the gecko toepad and its role for adhesion are still not entirely understood. The setal arrays are mainly composed of proteins, which provide the tissue rigidity needed for constant attachment and detachment. Commonly it is assumed that keratins are the major protein component of gecko setae.[9] The scaffold of proteins within the gecko adhesive system is held together by a matrix of biomolecules.

The structure of proteins within this architecture is expected to be closely related to the mechanical function, which is ease compliance under compression/bending and strong resistance under tension. The molecular structures of protein scaffolds are expected to be directly correlated to their mechanical properties.[10] This should be particularly true for gecko spatula which have a thickness of only approximately (5 to 10) nm, and thereby, the scaffold consists of only a few layers of proteins. How proteins assemble within spatulae to mechanically support such a thin functional structure is still an open question.

While the molecular organization of keratins in reptile skin, bird feathers, and other keratinous biological materials has been studied in detail,[11] there is still relatively little known about the structure of proteins within gecko setae arrays. In this study, we use near-edge X-ray absorption fine structure (NEXAFS) spectroscopy to investigate the keratin structure and their alignment within gecko adhesive pads (see Figure 1). In general, electron yield NEXAFS spectroscopy is a surface-sensitive method that probes the outermost (5 to 10) nm of a material.[12] In the present case, however, the probing depth is very close to the total spatula thickness of (10 to 15) nm;[7] therefore, the entirety of the spatula protein scaffold is probed (Figure 1).

In the NEXAFS experiment we probe two setal arrays in different orientations: one setal array (Figure 2A) is mounted with the adhesion direction parallel to the electric field plane (plane of polarization) of the incoming X-ray beam, and one is mounted perpendicular to the electric field plane of the X-ray beam. The geometry is illustrated in Figure 2B. The tissue samples have been attached to the sample holder with copper tape and then imaged by using a NEXAFS microscope. The advantage of using the imaging modality of NEXAFS spectroscopy in the context of tissue analysis is that the

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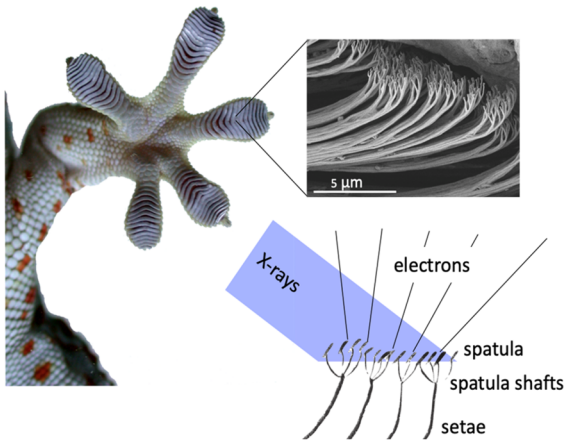

Figure 1. NEXAFS spectroscopy of gecko adhesive pads. Gecko footpads consist of adhesive setae arrays. The setae are terminated by spatula shafts. The spatulae at the end of the spatula shafts make the adhesive contact to the surface. In this study, the molecular structure of the spatula is probed through NEXAFS spectroscopy. Because of the geometry of the setae arrays, the experiment mostly probes the spatula structure while photoelectrons from the shafts and setae are absorbed or scattered by the tissue.

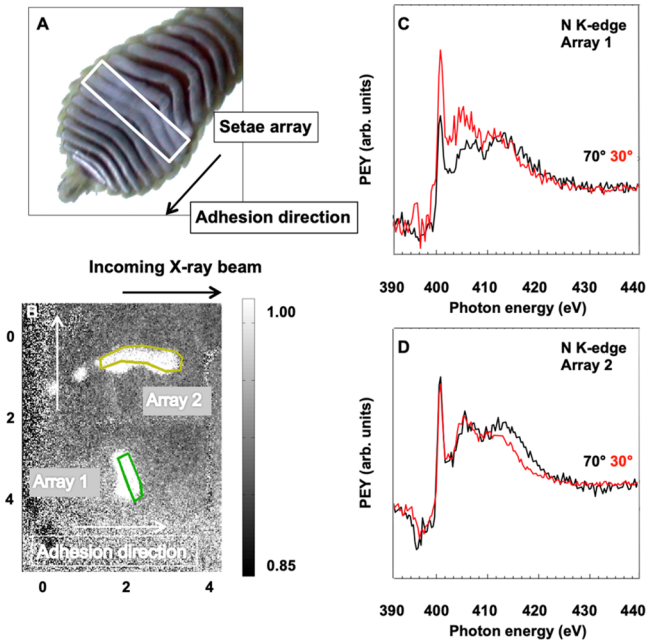

Figure 2. Angle-dependent NEXAFS spectra of electric field plane collected parallel and perpendicular to the setae adhesion direction. (A) The samples consist of setae arrays prepared from gecko footpads. (B) NEXAFS image representing the PEY across the nitrogen K-edge. The bright spots are two setae arrays mounted with the adhesion direction parallel and perpendicular to the incoming X-ray beam. The colored markings show the regions of interest used to extract the spectra. The distances on the x and y scale are in millimeters. (C) Pre- and postedge normalized NEXAFS spectra extracted from the ROI Array 1 for X-ray incidence angles of 70° and 30°. (D) Pre- and postedge normalized NEXAFS spectra extracted from the ROI Array 2 for X-ray incidence angles of 70° and 30°.

microscope is based on magnetic lenses for electron detection, which provides a method to probe curved, nonconducting samples without spectral distortion.[13] The instrument also allows to define exact regions of interest such that spectra for small objects can be readily extracted without possible contributions from the sample holder.

Figure 2B shows a NEXAFS image of the gecko tissue attached to vacuum copper tape. The image is based on the photoemission across the carbon K-edge scanned from 275 eV to 340 eV. The extracted pre- and postedge normalized nitrogen K-edge NEXAFS spectra can be seen in Figures 2C and 2D. The spectra shown are representative of a series of tissue sample, which have been investigated. While spectral shape and relative intensities can vary between specimen and individuals, the general spectral feature presented here are consistently found throughout the sample set. The spectra contain an amide π^* resonance near 401 eV, typically found in protein spectra, along with σ^* resonances above 405 eV, related to N−C and N−H containing groups in the backbone and side chains of proteins.[14,15] Certain lipids, such as sphingomyelin, can also contain amide species. Jain et al. have identified several lipid species at spatula surfaces.[16] To test in how far these specific lipids are contributing to the nitrogen spectra, we have performed reference measurements with samples, which were delipidized (see Figure S1). Samples with lipids removed showed no significant difference compared with the native gecko tissue, and therefore we can exclude a significant lipid contribution to the amide signal. The spectral features are related to proteins within the gecko tissue.

The spectra extracted from the region of interest covering the array mounted with the X-rays parallel to the adhesion direction are shown in Figure 2C for NEXAFS sample angles of 70° (near normal, electric field in sample plane) and 30° (glancing, electric field near perpendicular to the sample plane). The amide π^* resonance near 401 eV exhibits a pronounced angle dependence with a significantly higher signal intensity at 70°. Angle-dependent NEXAFS spectroscopy has been developed into a robust tool to probe the orientation of protein−amide bonds[15,17] by determining the orientation of amide orbitals within proteins.[12] The strong dichroism is typically related to β-strand-type structures. In helical or random-coil secondary structures, the amide bond, and thereby the respective transition diploe moments (TDMs), have a broad distribution of orientations, and only a minimal dichroism is observed.

This result strongly suggests that along the adhesion/friction direction the keratin proteins adopt a well-aligned β-strand structure. NEXAFS spectra show a positive dichroism, with the π^* intensity at 70° higher than at 30°, which is consistent with an upright orientation of the β-protein strands, which implies that the fibrils within the setae are aligned downward and parallel to the direction of the spatula shafts.

This view is supported by transmission electron microscopy (TEM) images of the setae. The images in Figure 3 clearly show the location of the fibrils within the spatula shafts: the dark, electron dense areas are related to protein fibrils, while the brighter areas are related to a cohesive matrix, which includes lipids and proteins. Note that the very bright spots in Figure 3A are coming from holes in the resin, where the seta was embedded and sectioned. In agreement with the NEXAFS results, the fibrils are aligned along the direction of the spatula shafts, all the way downward to the terminal spatula structures (see Figure 4 for an illustration).

Interestingly, angle-dependent spectra extracted from the setae array mounted with the adhesion direction perpendicular to the incident electric field plane of the X-ray beam did not show an appreciable dichroism for the amide π^* resonance

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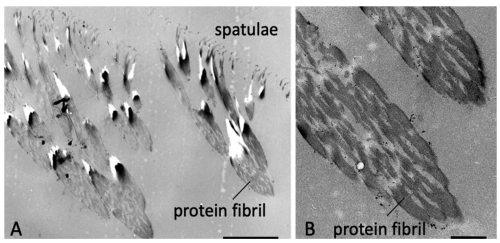

Figure 3. TEM images of gecko setae arrays. Scale bars: A, 10 μm; B, 1 μm. (A) β-Protein fibrils (electron dark areas) are visible throughout the setae structure, revealing branching pattern of spatula shafts and spatulae. The electron brighter areas are likely related to a matrix of biomolecules holding the fibrils together. (B) Detailed view of β-protein fibrils within the spatula shafts (oblique section).

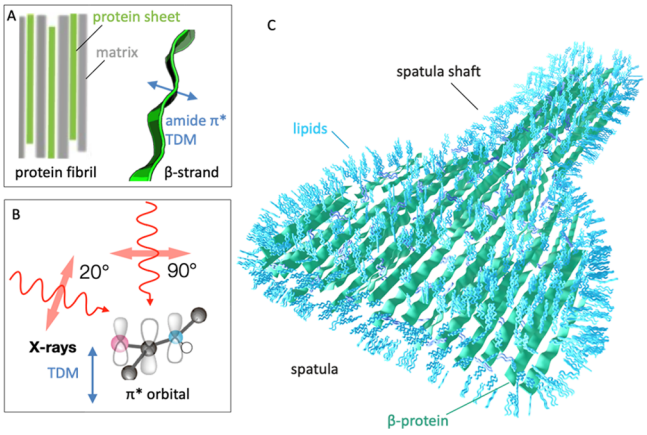

Figure 4. Model of protein assembly within the spatula. (A) The keratin is aligned within a matrix of biomolecules. The TDM of the amide π^* resonance is oriented perpendicular to the plane of the amide bonds. (B) Angle dependence of the intensity of the NEXAFS signal of the amide π^* orbital. For a geometry drawn in (A) a higher intensity is expected for β-strands oriented upright with respect to the surface of the footpads. (C) Model of the molecular structure of the spatula. β-strands are oriented along the direction of the spatula shafts.

near 401 eV (Figure 2D). This can be explained by the alignment of β-protein fibrils along the direction of adhesion. An upright and untwisted β-strand type structure would not generate a dichroism when probed perpendicular to the adhesion direction because the amide π^* transition dipole moments (TDMs) will be distributed over a wide range of orientations.[12]

The current picture of setal fibrils is mostly based on X-ray diffraction data for β-keratins within skin and feather tissue. Here, the fibrils consist of assemblies of twisted β-strand structures. A twisted β-strand will exhibit an angle dependence regardless of the azimuthal direction of the X-ray beam.[12] Because we observe a dichroism only along the adhesion/friction direction, the β-protein units are likely not twisted, at least not to the extend described in the keratin models. We can therefore conclude, that keratins within gecko spatula consists of a predominantly "flat" and untwisted structure (see Figure 4 for an illustration). Because the spatula thickness is about (10 to 15) nm, the "flatness" of the β-proteins in this region would be an adaptation to produce extremely thin and flat structures, which are essential for reliable contact formation with the variety of flat and rough substrate surfaces[8] and maybe even including molecular roughness of the substrate. Flat β-keratin microstructures are also known from the bird feather microhooks, which interlock individual barbs with each other. This provides structural integrity, while maintaining air permeability, of the feather vane.[18]

Jain et al.[16] have observed that lipids within setal tissue are more dynamic compared with lipids found in other gecko tissue. The highly aligned structure of the β-sheet protein fibrils could potentially promote lipid diffusion along the setae and spatula shafts and thereby allow swift replenishment of a lipid coating of the spatulae during locomotion.

From the mechanical point of view, a spatula should be adaptable and compliant, but on the other hand, it should be resistant against mechanical damages and wear. Therefore, one might expect high packing density and strong alignment of the flat, almost graphene-like molecules. The angle-resolved NEXAFS images provide evidence that the flat keratin structures are well-aligned with the adhesion direction of the setae, which provide additional mechanical stability to the setae across different length scales of setal architecture.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpclett.2c00004.

Details of experimental methods and additional data (PDF)

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Notes

The authors declare no competing financial interest.

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