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Bloodworm's Way With Copper Likely Provides Paradigm for New Materials

The researchers determined that copper also occurs in non-mineral form in the bloodworm jaw where it may act as a structural element in cross-linking long chains of fibrous proteins. According to the authors, "The marriage of protein with copper mineral as well as with bound copper ions is an intriguing concept per se but may also serve as a design prototype for new materials that need to be hard, lightweight, and durable."

In addition, they found the non-mineral form of copper on the surface of the jaw canal through which venom is injected. That copper may be acting as a catalyst that activates venom being discharged by the worm into its prey.

The authors include University of California Biology Professor Herbert Waite and Chemistry and Materials Professor Galen Stucky. First author Helga Lichtenegger, who was trained in Austria as a physicist, approached Waite and Stucky about working with them on a research project. Waite dipped into a repository of promising projects, which he calls his "orphans," and suggested Glycera dibranchiata-better known to fishermen as the common bloodworm, "favorite bait for certain kinds of fish," according to Waite.

"Back in 1980 two British investigators reported high levels of copper [up to 13 percent by weight] and zinc in the jaws or fangs of two rather common species of polychaete worms," said Waite, who is an expert on mussels and the fibers mussels make to attach themselves to stones in inter-tidal basins. The mussel fibers consist largely of proteins, but one percent of their weight content is metallic-copper, zinc, or iron. "I have been trying to understand what role these transition metals play in those fibers," said Waite. "We have discovered that if we leach out the metals, the structure is weaker."

Because of Waite's long-standing interest in metals in mussel fibers, he had earmarked the 1980 report on the copper and zinc in blood- and clamworms as intriguing. Funding sources, however, failed to share Waite's enthusiasm for the worms. Supported by the Austrian Science Fund (Fonds zur Foerderung der wissenschaftlichen Forschung, FWF), Lichtenegger joined Waite and Stucky as a postdoctoral fellow at UCSB to study the worms despite the absence of specific funding for the project.

"We had an enormously good time," said Waite. "It is really nice once in a while to do whatever your intellect dictates. We didn't have enough preliminary information to justify independent funding for the project. It just seemed an interesting orphan."

"Orphan" though the genesis be, the progeny of the research are not. In addition to providing a possible paradigm for a new material system, other possible applications include, for instance, drug delivery. This research is an example of biomimetics, whereby an organism's biomolecular strategies for adaptation are studied and then employed to make new materials and devices. The Science paper focuses on the first step of determining and understanding the organism's biomolecular structures and raises even more tantalizing questions about them than it answers.

A skilled x-ray scattering specialist, Lichtenegger and another postdoc in Stucky's group in the UCSB Department of Chemistry and Biochemistry, Michael Bartl (also an author on the Science paper), employed a full arsenal of high resolution imaging techniques to study the worms.

"The thing that really galvanized our attention," said Waite, "was Helga's detection of copper biomineral at the bloodworm's jaw tip." The 1980 findings gave no indication that the copper was associated with a mineral.

As the authors state at the outset of the Science paper, "Biomineralization is a major strategy for tissue hardening and manifests an astonishing diversity of bioceramic structures with exquisite microarchitectures that have specially adapted physical properties."

Ten to 15 inches long, the bloodworm comes equipped with a proboscis not ordinarily visible. The proboscis is hydrostatically ejected-i.e., water pressure is used to extend it fast the way a paper noisemaker unfurls when blown. At the end of the fleshy red-pink proboscis are four black jaws that resemble in shape the thorns on a rose bush. The jaws at the end of the proboscis grab and bite prey the way four-pronged devices at the end of long poles are used to grasp and to pluck products from the high shelves of a grocery store. In contrast to the clamworm, the bloodworm's jaws are hollow, like syringes, and are used to conduct venom into the prey.

The researchers found that the jaw tip exhibits the ordered crystalline structure of the copper-based biomineral atacamite [Cu2(OH)3Cl], the first discovered instance of a copper biomineral in a living organism.

"Copper is very nasty stuff," said Waite. "Though needed in trace amounts for enzymatic processes, copper in greater quantities is toxic. Our research leaves us with questions. How does the worm collect all this copper and concentrate it in a safe form until it is invested in these jaws? What kind of biochemical pathways has this organism evolved to prevent copper toxicity, and what kind of protein carriers are there that bring it to the site of mineralization?"

The bloodworm lives in the gravel of marine sediments and smells out its prey, explains Waite. "The worm is going to miss its prey a fair number of times," he said. "And that means that its jaws are being abraded by gravel. So they need to be made of more robust material than the jaws of the clamworm, which is a scavenger and lives by grabbing things that have died."

The researchers found that the bloodworm's unmineralized copper as well as the copper biomineral is concentrated in the first half millimeter of the jaw. They discovered that the copper biomineral is organized in nanostructured fibers (about 50 nanometers in diameter) within a protein matrix, and that the "fiber orientation within the tip is roughly parallel to its outer shape," according to the paper entitled "High Abrasion Resistance With Sparse Mineralization: Copper Biomineral in Worm Jaws."

Through a complex procedure called "nanoindentation" (performed by the paper's author Thomas Schöberl of t he Erich Schmid Institute for Materials Science, Leoben, Austria), the researchers mapped hardness (H) and stiffness (E) with composition in the bloodworm jaw. They then determined that both hardness and stiffness increased with increasing mineral content.

"The question as to why the bloodworm selects copper for its jaws is very puzzling; most other organisms mineralize with calcium phosphate/calcium carbonate," said Waite. "And in all those other calcium-based biting, chewing, fighting structures, the mineral to protein ratio is more like 10 to 1. That's true for enamel (the biting surface of teeth) and for the tips of serpent fangs. Most other organisms make bioceramics in which a "gossamer" protein scaffold is mineralized with ordered arrays of inorganic components with certain geometries such that the combination provides for wear resistance. But this worm turns that paradigm on its head. It makes a structure that is 10 parts protein and one part mineral and exploits that structure for wear resistance. If you want to make something hard, how do you do it with a preponderance of protein? We think the worm does it by adding copper!"

UCSB Materials Professor Frank Zok helped to clarify the cleverness of that strategy exhibited by the bloodworm by suggesting a more mathematically complex understanding of wear resistance than the team had been employing. Relying just on hardness and stiffness values had led the team to conclude that the copper biomineral does not compare favorably with other bioceramics. Sch?berl, however, had noticed that the h ardness to stiffness ratio in bloodworm jaws was higher than in other known mineralized tissue. Zok suggested that this was indicative of a high abrasion resistance, commonly understood as equal to hardness to the three halves divided by stiffness [H3/2/E].

"With the worm," said Waite, "it is that quotient that is revealing rather than individual hardness and stiffness measurements. So when you look at that quotient, the wear resistance value is very close to the best materials we can make."

But high values for H and E also correlate with the presence of copper, even where the copper is not in mineral form. Lichtenegger's analysis of the distribution of copper in the bloodworm jaw suggested that about half the copper was in a biomineral form and half in the form of copper aggregates. That finding particularly interested Waite.

He said, "I know from my research that the proteins in invertebrate jaws and particularly in the jaw tips are not complicated. They have generally just two types of amino acids, glycine and histidine. These two amino acids probably repeat in a precise chainlike sequence. In this respect it is one of Nature's closest analogues to synthetic polymer molecules. I expect that the copper cation in the bloodworm jaw plays a role in the cross-linking of these amino acid chains."

So the copper contributes two ways--in mineral form and in cross-linking--to make the jaw material strong and tough.

Finally, the authors raise the possibility "that copper may mediate the activation of venom during injection."

"Storing venom in non-toxic form that is being catalytically activated as it passes through this syringe may," suggested Waite, "afford us a model for delivering an unstable chemical by activating a stable form of it as part of the parting shot." Among the applications he envisions is a system for delivery of unstable drugs-those with a short shelf life. "We may be able to synthesize the drug in a precursor form and have that last important step done as part of delivery."

Stucky, who is an expert on the molecular assembly of organic and inorganic species into three-dimensional, hierarchically patterned materials and whose research group has extensively studied marine organism biomineralization, is a participant in the California NanoSystems Institute (CNSI). Waite is a participant in UCSB's Marine Science Institute (MSI).

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