Biological Materials Chemistry

DOI: 10.1002/anie.201003171

The Iron-Fortified Adhesive System of Marine Mussels**

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materials science · mechanical properties · proteins · surface chemistry · transition metals

Look to the seas and you will find a variety of fascinating materials residing within, from shells to reefs to adhesives. Barnacles, giant clams, limpets, tube worms, star fish, sea cucumbers, and kelp are examples of the many organisms that produce adhesives and cements to aid survival. These species can affix themselves to rocks and thereby build communities that deter predators, aid reproduction, and decrease buffeting of the turbulent intertidal zone. The adhesive system used by blue mussels has become the focus of much attention for ongoing biomaterials characterization (Figure 1). This attachment system is called a "byssus" or "beard", owing to the threads fanning out from between shells (Figure 1). Adhesive plaques terminate each thread and make contact with the surface. Both the threads and the plaques are predominantly protein, with high levels of the unusual 3,4-dihydroxyphenylalanine (DOPA) residue present (Figure 1). An amazing story is now emerging in which iron has been found to bind these DOPA proteins for two separate, but related, purposes: to improve the mechanical performance of the threads and to bring about formation of the adhesive plaques.

Mussel threads are an extensible and hard material comprised of two distinct regions: an inner core and a thin outer coating. Similarities to human hair are both visual and biochemical. Crystalline protein domains of collagen make up the thread core, and elastin and fibroin domains are also present. DOPA proteins form the thin (2–5 μm) outer cuticle and make this coating approximately 5–10 times harder and stiffer than the core. The first indication that metals may be influencing materials properties of the threads was the observation that treatment with chelators (e.g., ethylenediaminetetraacetic acid) resulted in softening and changes to the stress–strain behavior.[1] Native threads are able to recover from stress.[1] Upon chelation treatment, this "self-healing" property was lost, but it was recovered when metal ions were returned, which showed that any relevant metal–protein interactions are reversible.[1] Examination of the cuticle

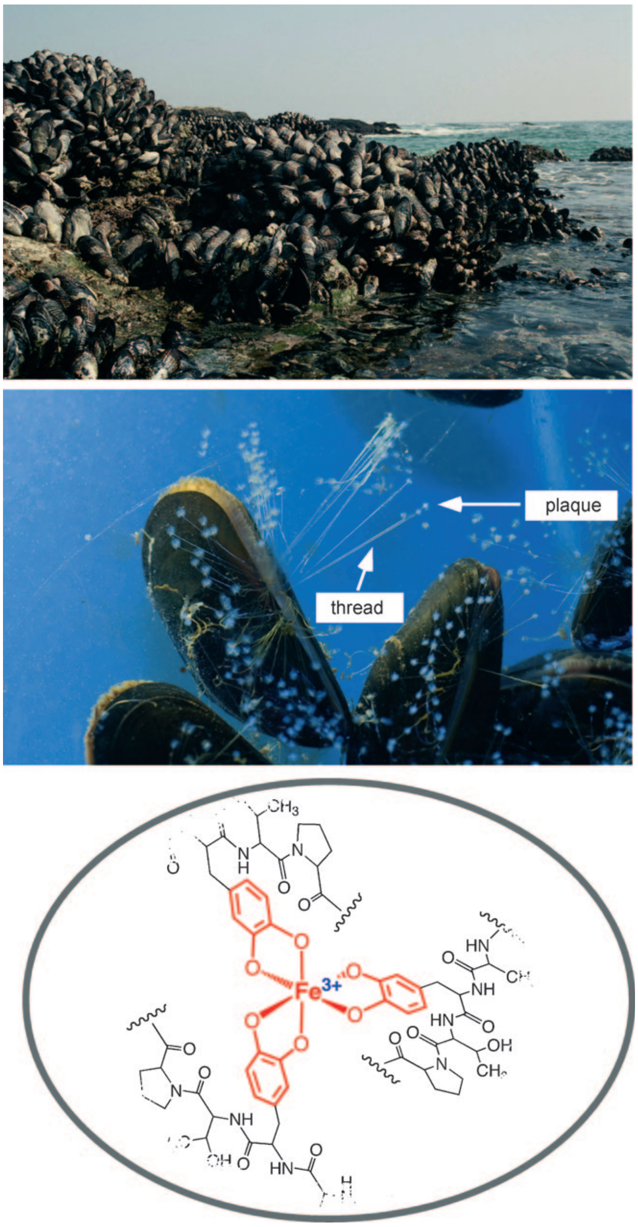

Figure 1. Top: A community of mussels affixed to rocks. Middle: Mussels adhering to glass. The picture shows their byssal adhesive system consisting of threads and plaques. Bottom: An $[Fe(dopa)_3]$ complex.

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[**] Support from the Office of Naval Research and the National Science Foundation is appreciated.

microstructure revealed the presence of granules bound within a larger matrix.[2] This heterogeneous structure probably exists for the purpose of discouraging crack propagation.[2]

Next, it was found that iron and DOPA are colocalized in the cuticle but not the thread core.[3] Despite this high iron concentration, no mineralization was seen.[3] Most recently, confocal Raman spectroscopy and mapping were used to show that a higher concentration of Fe–DOPA interactions were present in the granules than in the bulk matrix of the thin cuticle.[4] This progression of studies has been constructing a picture of how mussel threads display the seemingly opposed properties of being simultaneously hard and extensible.[1–4] Iron–DOPA interactions harden the material, whereby the reversible formation of such bonds permits extension and recovery after stress.[4]

These recent and exciting results with mussel threads provide unprecedented insight into how metal–biomolecule bonding can influence materials properties. More broadly, these conclusions fit within the growing body of studies showing that metals can be used to enhance the mechanical performance of biological materials. Since the 1970s, spider fangs, insect mandibles, chiton teeth, and marine-worm jaws have all been shown to localize manganese, iron, copper, or zinc for the purpose of hardening organic structures.[5] Another recent discovery that is receiving attention concerns a deep-sea gastropod: This snail stays safe from predators by creating a strong shell constructed from an organic matrix hardened with iron sulfide granules.[6]

Iron may improve the hardness and extensibility of mussel threads, but their structure is likely to still exist without the metal ions. A contrasting picture of adhesive plaques is developing according to which iron is essential to the very formation of this material. Plaques are a cross-linked matrix of DOPA proteins with metals such as iron, zinc, copper, and manganese at concentrations significantly higher than those found in water around the animals. Metal ions bind tightly to the catechol-like side chain of DOPA, so it is easy to imagine that metal–DOPA bonding is taking place.

Much is now being discovered about the bonding motifs and influences of metals on adhesion. When DOPA adhesive proteins were extracted from mussels and treated with a library of potential cross-linkers, such as metal ions and oxidants, iron(III) stood out as the bioavailable species most able to bring about cross-linking and the formation of a hardened material.[7] The investigation of adhesive plaques, extracted protein treated with Fe^{III}, and Fe^{III}–peptide complexes by multiple spectroscopic methods revealed three key insights: the presence of $[Fe(dopa)_3]$ complexes, reactions of $[Fe(dopa)_3]$ with O_2, and the formation of organic radicals.[8] The generation of mussel adhesive can thus be attributed to a multistep mechanism beginning with Fe^{III} templating three DOPA-containing protein chains into $[Fe(dopa)_3]$ complexes (Figure 1).[8] Iron reduction ($Fe^{III} \rightarrow Fe^{II}$) and DOPA oxidation to a semiquinone occur simultaneously. Reaction of the semiquinone with O_2 then generates the protein-based radicals.[8] Subsequent radical–radical coupling provides cohesive protein–protein cross-links in the bulk.[8] Adhesive bonding to the surface can result from radical–surface coupling.[8] An atomic force microscopy study has also shown that adhesive interactions are formed when the "reduced" (i.e., not oxidized) form of DOPA binds directly to metal surfaces.[9]

The initiation of oxidation to form protein-based radicals is reactivity typically reserved for enzymes. However, in mussels, enzyme chemistry appears to occur without the enzyme. In this way, the versatility of reactions involving iron, DOPA, and oxygen may both enable the formation of the material and contribute to adhesive performance. Somewhat similar chemistry with small molecules is responsible for the dark colors of defensive ink squirted by an octopus, melanin pigments, and iron gall pen ink, which has been in use for centuries.

Combinations of metal ions and oxidants were found to contribute synergistically to the cross-linking of DOPA proteins.[10] Simple oxidants, such as H_2O_2, can enhance the cross-linking of metal ions, such as Fe^{II}, to a greater degree than was found from the sum of the effects of the individual reagents.[10] This result raises the possibility that an oxidant may be coadministered with iron when a mussel wants to adhere.[10]

Bulk adhesion data for DOPA proteins are now becoming available.[11] The addition of Fe^{III} enhanced the bond strength of extracted DOPA proteins when these proteins were used to attach strips of pig skin. However, the Fe/DOPA ratio was important: With too little iron, adhesion was not optimized. With too much iron, extensive cross-linking took place at the expense of adhesive performance.[11] How iron can balance cohesion with adhesion is one of the many facets of mussel glue still waiting to be uncovered.

Characterization of the mussel adhesive system has shown two unique roles for iron. In the threads, Fe–DOPA bonds improve mechanical performance. In the case of plaques, $[Fe(dopa)_3]$ complexes are responsible for formation of the material and also contribute to adhesive bonding. We cannot help but wonder if these principles may also be at play in other biological materials. Even more exciting is the prospect of discovering new correlations between chemistry and materials properties in nature. As we come to understand the blueprints used by biology, this knowledge can then be applied to the design of high-performance synthetic materials, such as self-healing polymers, adhesion-fighting antifouling coatings, and wet-setting biomedical glues.

Received: May 26, 2010

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