Zuschriften

Marine Muscheln heften sich mit einem proteinbasierten Klebstoff an Oberflächen. Nach Untersuchungen am „Muschelkleber“, dem extrahierten Protein und Peptidmodellen beruht die Härte dieses verbreiteten biologischen Materials auf einer Vernetzung durch [Fe(dopa)$_3$]-Komplexe. Mehr zu diesem Thema entnehmen Sie der Zuschrift von J. J. Wilker et al. auf den folgenden Seiten.

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Marine Chemistry

Metal-Mediated Cross-Linking in the Generation of a Marine-Mussel Adhesive**

*Mary J. Sever, Jaime T. Weisser, Jennifer Monahan, Shalini Srinivasan, and Jonathan J. Wilker**

Mariners have been contending with the tenacious holdfasts of barnacles and mussels for over 4000 years. These protein-based adhesives exhibit striking materials properties still unmatched by human technology. Such hardened matrices are formed by extensive cross-linking of protein precursors.[1–3] This cross-linking approach to biomaterial synthesis is shared by glues from mussels, limpets, and kelp, cements from barnacles, oysters, and polychaete worms, coral skeletons, and skate egg shell cases.[2,3] In none of these systems, however, is there available a detailed picture of the bonding schemes employed for material construction. For mussel adhesive plaques (Figure 1), posttranslational incorporation of 3,4-dihydroxyphenylalanine (DOPA) into the protein (Scheme 1) is essential for subsequent cross-linking and proper adhesion.[1–3] Other interesting properties of the mussel glues include a transition-metal-ion content (e.g., copper, iron, and zinc)[4,5] up to 100 000 times that found in open ocean waters.[6] Recently we have shown that metal ions, in particular Fe^{III}, bring about curing of adhesive precursors extracted from mussels.[7] To gain insights on bonding of marine adhesives, we present data on mussel glues and related systems indicating that metal–protein interactions participate in the generation of these materials.

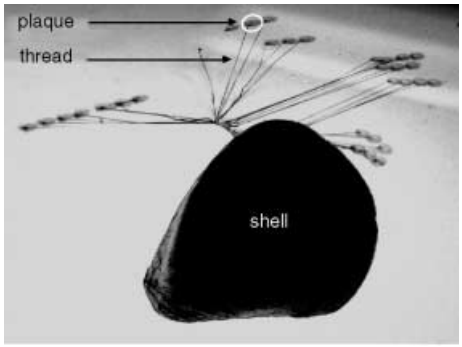
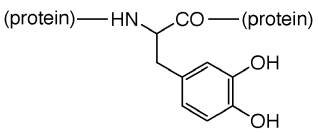


Figure 1. Photograph of a marine mussel adhering to a glass sheet.



Scheme 1. Protein-bound 3,4-dihydroxyphenylalanine (DOPA).

Common blue mussels (*Mytilus edulis*) were collected from coastal Maine and placed on glass sheets in salt water tanks (deionized water and Marine Environment salt) at 6 °C. After deposition of adhesive (Figure 1), mussels and threads were cut free, the adhesive plaques were scraped from the glass, washed with deionized water, and dried under vacuum. Collected plaques were examined by electron paramagnetic resonance spectroscopy (EPR, Figure 2a) at 15 K. This spectrum showed the conspicuous presence of high-spin Fe^{III} centers ($g = 4.239$, 1531 G; $g = g$ factor) and an organic radical ($g = 1.997$, 3249 G), possibly accompanied by weak signals for Cu^{II} or low-spin Fe^{III} centers (2615, 2734, 2904, 3070 G).[8,9]

The DOPA-containing proteins Mefp-1 and Mefp-2, soluble precursors to cured mussel adhesive,[2,3] were extracted from mussel feet prior to cross-linking.[10] A frozen solution of mussel precursor proteins in 10% glycerol showed neither a significant Fe^{III}-ion signal nor any evidence of an organic radical when examined by EPR spectroscopy (Figure 2a). Addition of aqueous iron ions (e.g., $Fe(NO_3)_3$ or $FeCl_3$ at 500 mM, pH ≈ 1.5) to a solution of the soluble adhesive precursor proteins (DOPA concentration of 0.5 mM, pH ≈ 1.5) brought about immediate precipitation of a beige solid which was isolated, washed, and dried. Examination of this solid by EPR spectroscopy displayed signals for an Fe^{III} ion and an organic radical, which showed this spectrum to resemble that of adhesive plaques from live mussels (Figure 2a). Aqueous solutions of $Fe(NO_3)_3$ or $FeCl_3$ showed EPR spectra distinct from all other samples. The radical found in plaques and the iron–protein solid thus appears to arise from Fe^{III}-ion-dependent oxidation of the protein. The highly reactive properties of radical species may provide a pathway to organic cross-links such as a di-DOPA moiety[11] or enable coupling to surfaces and formation of adhesive bonds.

Adhesive plaques were pressed into a KBr pellet for examination by infrared spectroscopy (IR, Figure 2b). Bands for amide I and II (≈1656 and ≈1534 cm^{-1}) were visible along with a cluster (1458–1439 cm^{-1}) attributable to DOPA.[12] Soluble adhesive precursor protein[10] was lyophilized into a colorless solid and investigated by IR spectroscopy (Figure 2b). Similar features were observed when compared to the plaques, however, the protein showed one prominent DOPA band (1456 cm^{-1}). The iron–protein solid exhibited a DOPA band split to 1456 and 1437 cm^{-1}. Such splitting is indicative of metal binding as exemplified by the case of enterobactin, a tris (1,2-dihydroxybenzene) ("catechol") ligand employed in bacterial iron transport.[13] The enterobactin catechol band at 1460 cm^{-1} splits into 1460 and

[*] M. J. Sever, J. T. Weisser, J. Monahan, S. Srinivasan, Prof. J. J. Wilker
Department of Chemistry, Purdue University
560 Oval Drive
West Lafayette, Indiana 47907-2084 (USA)
Fax: (+1) 765-494-0239
E-mail: wilker@purdue.edu

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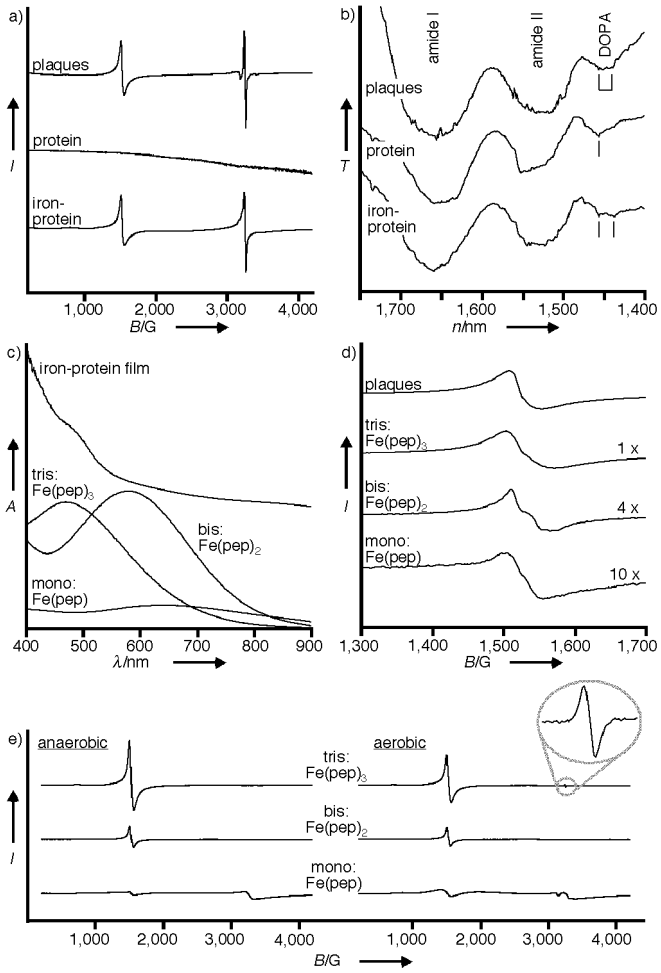

Figure 2. Spectroscopic characterization of marine mussel adhesive plaques and related systems: a) EPR spectra of intact mussel plaques, adhesive precursor protein, and precursor protein precipitated from solution with $Fe(NO_3)_3$. b) IR spectra of plaques, lyophilized precursor protein, and iron–protein solid. c) UV/Vis spectra of an adhesive film prepared from precursor protein reacted with $Fe(NO_3)_3$ and solutions of mono, bis, and tris Fe-AdopaTP peptide complexes, $[Fe(pep)_n]$. The solid spectrum was obtained by overlapping four films. d) EPR spectra of plaques with tris, bis, and mono $[Fe(pep)_n]$ peptide complexes. Vertical scale on spectra were adjusted as indicated to aid comparisons. e) EPR spectra of tris, bis, and mono $[Fe(pep)_n]$ complexes prepared anaerobically and aerobically. An expansion is provided for the radical region of aerobic $[Fe(pep)_3]$, the only sample to exhibit a radical.[15]

1440 cm^{-1} upon binding iron.[13] Thus, the single DOPA band for adhesive precursor protein contrasts with the split bands of the iron-protein solid and whole plaques, which indicates metal binding in the latter two samples.

UV/Vis absorption spectroscopic studies with the DOPA-containing peptide models Ac-Ala-DOPA-Thr-Pro-$CONH_2$ ("AdopaTP") and Ac-Asn-DOPA-Arg-Gly-$CONH_2$ ("NdopaRG")[14] showed strong binding of the DOPA catechol-like side chain to Fe^{III}, but no interaction with Cu^{II}, Zn^{II}, or any other divalent transition-metal ion.[15] In addition to bringing about a focus on iron chemistry, such model studies provide complexes for spectral comparisons with the whole plaque and protein systems. Although poor optical quality of whole plaques prevented us from obtaining a suitable UV/Vis spectrum, the reaction of mussel protein[10] with $Fe(NO_3)_3$ on silanized glass yielded a uniform adhesive film that exhibits absorption at $\approx$470 nm (Figure 2c).[15] A comparison with UV/Vis spectra of peptide models indicated $[Fe(DOPA)_3]$ coordination within this material (Figure 2c). Control experiments with protein alone, $Fe(NO_3)_3$ alone, or bovine serum albumin with $Fe(NO_3)_3$ did not form an adhesive film or show visible-light absorption.

EPR spectra of anaerobic $[Fe(AdopaTP)_n]$ models in 10% glycerol displayed Fe^{III} signals for mono ($n=1$), bis ($n=2$), and tris ($n=3$) complexes, with splitting observed in the bis, $\{Fe(AdopaTP)_2\}$, case ($g\approx4.205$, $\approx$1538 G, Figure 2d). This splitting was also present in the EPR spectra of bis, $\{Fe(L)_2\}$, models prepared with the ligands L = catechol or NdopaRG, but not in tris, $[Fe(L)_3]$, complexes.[15] A lack of such splitting in the EPR spectra of whole plaques and iron–protein solid indicates that bis, $\{Fe(DOPA)_2\}$, cross-links are not present in these samples (Figure 2d). A mono coordination environment in the plaques and iron–protein solid is also unlikely owing to an inability to bring about protein–metal–protein interactions.

When $[Fe(AdopaTP)_n]$ models were prepared in air, a radical signal was observed ($g=1.989$, 3251 G) in the EPR spectrum of the tris, $[Fe(AdopaTP)_3]$, complex (Figure 2e).[15] No evidence was seen for radical formation in the mono, $\{Fe(AdopaTP)\}$, or bis, $\{Fe(AdopaTP)_2\}$, species in air.[15] Similar results were found when the models were prepared from solutions with excess peptide. Observed peptide oxidation by oxygen and iron from an $[Fe(DOPA)_3]$ complex, as opposed to the bis or mono analogues, is consistent with a greater degree of Fe^{III}- and less Fe^{II}-ion character in the tris coordination environment.[16] Radical generation in the aerobic $[Fe(AdopaTP)_3]$ complex coincided with an Fe^{III}-ion signal ($g=4.209$, 1537 G) of decreased intensity relative to the anaerobic sample (Figure 2e). These data suggest the occurrence of a redox reaction in which ligand oxidation is accompanied by a $Fe^{III} \rightarrow Fe^{II}$-ion reduction. Such intramolecular redox processes are known to occur in metal–catecholate systems.[17]

Although enterobactin and other siderophores have been known for quite some time,[18] metal–catecholate interactions are becoming increasingly conspicuous in biology. Recent findings on the extradiol[19] and intradiol[20,21] catechol dioxygenases may provide further insights on adhesive synthesis. A proposed mechanism for the intradiol catechol dioxygenases begins with an Fe^{III}–catecholate followed by valence tautomerism to a Fe^{II}–semiquinone.[20,21] Subsequent reactivity with oxygen yields a ligand-based radical species for further chemistry.[20,21]

In summary, EPR and IR spectroscopies provide evidence for metal–DOPA interactions in mussel adhesive plaques. An adhesive film was produced by iron curing of extracted protein. Iron in the presence of air oxidized both extracted protein and peptide models to yield products that contain organic radicals. More specifically, insights upon the coordination environment of iron were gained from EPR data in which the Fe^{III} of plaques did not resemble bis, $\{Fe(DOPA)_2\}$, bonding. The adhesive film prepared by reaction of iron and

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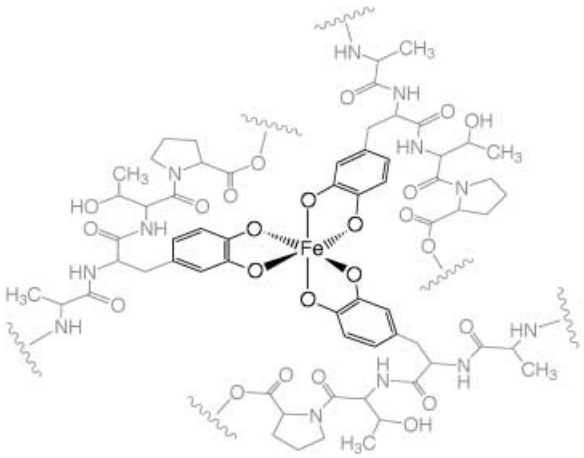
protein displayed a UV/Vis spectrum similar to that of the tris, [Fe(AdopaTP)$_3$], model. Finally, only the tris, [Fe(AdopaTP)$_3$], complex underwent oxidation to yield a radical upon exposure to air. When coupled with results showing that Fe^{III} brings about curing of adhesive extracts more than other ions,[7] these data implicate iron to be the key reagent in protein cross-linking for adhesive synthesis. Thus, we propose that the iron center in mussel plaques cross-links three DOPA residues as shown in Scheme 2. Subsequent reaction of this



Scheme 2. Proposed mussel adhesive metal-protein cross-link.

[Fe(DOPA)$_3$] and oxygen brings about protein oxidation, generation of a reactive radical species, and a potential means of coupling to a surface for adhesive bond formation. Mussel glues present the first identified case in which transition metals play an integral role in the generation of a noncrystalline biological material. The impressive properties derived from metal-protein interactions may prove to be a prevalent theme in marine biomaterials such as those of coral reef structures, kelp adhesives, and barnacle cements.

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