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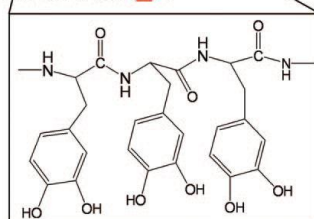
**Technical Memorandum No. MERL-2013-43**

Review of Mussel Adhesion Mechanism and Scoping Study

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Technical Memorandum No. MERL-2013-43

Review of Mussel Adhesion Mechanism and Scoping Study

U.S. Department of the Interior
Bureau of Reclamation
Technical Service Center
Materials Engineering and Research Laboratory
Denver, Colorado   **August 2013**

BUREAU OF RECLAMATION
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Materials Engineering and Research Laboratory, 86-68180

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Review of Mussel Adhesion Mechanism and Scoping Study

_____   8-28-13

Prepared by: Bobbi Jo Merten, Ph.D.   Date
Chemist, Materials Engineering and Research Laboratory, 86-68180

_____   8-23-13

Checked by: Allen D. Skaja, Ph.D., PCS   Date
Chemist, Materials Engineering and Research Laboratory, 86-68180

_____   8-27-13

Editorial Approval: Sharon Leffel   Date
Technical Editor, Economics, Planning, and Technical
    Communications Group, 86-68270

_____   9/4/13

Technical Approval: David S. Tordonato, Ph.D., P.E.   Date
Materials Engineer, Materials Engineering and Research Laboratory,
    86-68180

_____   9-4-13

Peer Review: William F. Kepler, Ph.D., P.E.   Date
Manager, Materials Engineering and Research Laboratory, 86-68180

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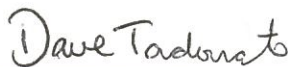
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ACRONYMS AND ABBREVIATIONS

Dopa   amino acid protein, 3,4-dihydroxyphenylalanine

KCl   potassium chloride

kDa   atomic mass unit, kilodalton

M   molar concentration, molar

MALDI-TOF   matrix-assisted laser desorption/ionization-time of flight

MAP   mussel adhesive protein

Mefp   marine blue mussel (*Mytilus edulis*) foot protein

mfp   mussel foot protein

mg   milligrams

Reclamation   Bureau of Reclamation

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Figures (continued)

EXECUTIVE SUMMARY

The funding for this project was provided under the Science & Technology Program, project #7419. The original proposal was a small laboratory scoping level experiment to investigate the feasibility of using mussel adhesive to form a protective coating over steel surfaces, which could benefit the corrosion-protective as well as the underwater-cure coatings industries. It was challenging to locate a vendor for supplying mussel proteins, and it was concluded that the materials were cost-prohibitive for our experimental interests.

Several significant advances in the understanding of the mussel adhesion mechanism had occurred since the Bureau of Reclamation's (Reclamation) previous investigation (Skaja 2011). The state-of-the-art is provided here. Sections of this report have been forwarded for publication within a chapter of the book entitled Biology & Management of Invasive Quagga/Zebra Mussels in the Western United States (Skaja et al. 2014, *in press*).

Zebra and quagga mussel research is important to identify methods for mitigating these invasive species that now inhabit the Western United States waterways where Reclamation operates hydroelectric power facilities and associated equipment. These mussels attach to almost any underwater substrate, repopulate under a wide variety of conditions, and are uncontrolled by native predator species.

Mussel animals occupy a shell, with the exception of an exogenous foot, which can be extended into a body of water. The mussel foot is used to select and prepare a surface for attachment. It is subsequently placed against the surface, and the process begins to build a plaque and thread. The plaque (disc-shaped pad) attaches the organism directly to a surface, and the thread (thin filament) connects the plaque to the mussel body. Within 5 minutes, the foot is lifted to reveal the newly formed thread/plaque structure that acts as an anchor for the mussel. This process is repeated to form multiple attachments. The thread/plaque structures are collectively referred to as the mussel byssus.

This document reviews the attachment mechanism of the mussel to its chosen substrate; therefore, it focuses on the mussel plaque. Plaque formation occurs in an area of the mussel foot called the distal depression. There are six mussel foot proteins (mfp) utilized in this process. Researchers have numbered and characterized each for their role in mussel byssus formation. Mfp-1 is a cohesive protein and forms the plaque and thread outer sheath. Mfp-3 and -5 adhere directly to the substrate. Mfp-6 is cohesive and binds these proteins together. Mfp-2 is also cohesive and binds most of the bulk of the plaque matrix together. Mfp-4 is cohesive and adjoins the plaque to the thread.

Adhesion of the mussel plaque is achieved through both adhesion and mechanical interlocking. The amino acid protein 3,4-dihydroxyphenylalanine (Dopa) is key

to the wet adhesion. The proteins 4-hydroxyphenylalanine (tyrosine) and (S)-2-amino-3-(phosphonooxy)propionic acid (phosphoserine) also participate in adhesion. The proteins form bidentate hydrogen bonding, metal/metal oxide coordination, and oxidative crosslinking. Mfp-3 and -5 have the highest concentration of Dopa of all foot proteins at 20–25 and 27 percent, respectively. They are also very low molecular weight, which likely causes them to have greater mobility. Figure ES-1 illustrates the mussel plaque and organization of the various foot proteins as supported by current theory.
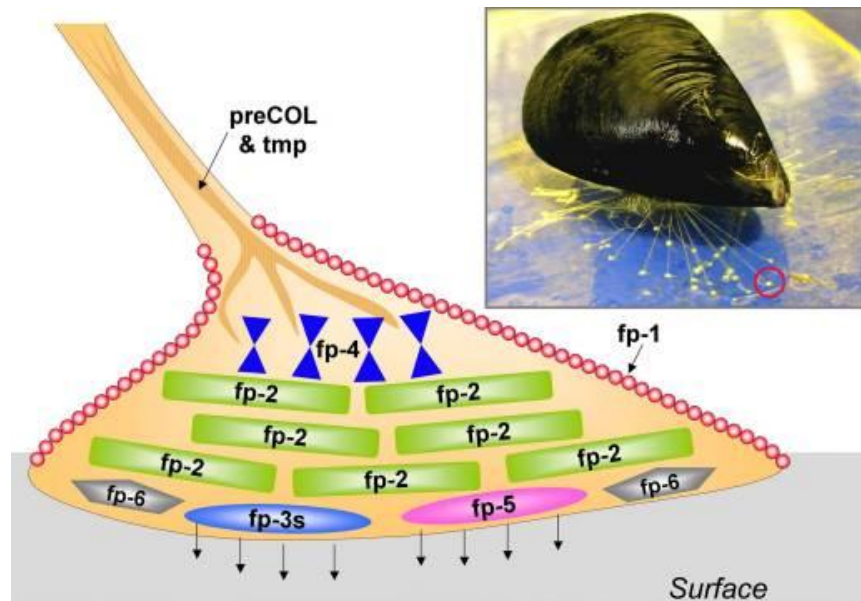
Figure ES-1.—Organization of mussel foot proteins within the mussel plaque. (Reprinted from Hwang et al., 2010)

Direct observation of mussel attachment was recently coupled with matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry to identify the foot proteins as they appear in byssal plaque and thread formation. This experiment verified that the unique sequence of plaque formation begins with co-secretions of mfp-3 and -6. Mfp-5 is also detected during the first minute. The mussel foot isolates and facilitates a micro-environment during plaque formation, and it is has a pH of ~5 and an ionic strength 0.1 M at the initiation of the process. Mfp-6 is utilized by the mussel to control the redox cycling of various amino acid proteins. The thiol-containing Mfp-6 reduces the Dopa, which has spontaneously oxidized (Dopa quinone – cohesive properties only) to ensure strong adhesion, and is the key to achieving the proper balance of adhesive and cohesive bonds within the mussel plaque and at the plaque/substrate interface.

PREFACE

The funding for this project was provided under the Science & Technology Program, project #7419. The original proposal for this work was ambitious and anticipated a literature review on the mussel proteins as well as a small laboratory scoping level experiment to investigate the material's ability to form a smooth, uniform coating over prepared steel surfaces. It was hypothesized that such a coating would exhibit strong underwater adhesion and excellent corrosion resistance due to this adhesion. A matured product stood to advance the corrosion-protective coatings as well as the underwater-cure coatings industries.

During the onset of this project, it was challenging to locate a vendor for supplying mussel proteins. One vendor provided a price quote of $1,600 for 100 milligrams (mg) (MAPTrix^{TM}, Kollodis Biosciences, Inc.). An additional company marketed their material online at $1,500 for 15 mg (Cell-Tak^{TM}, BD Biosciences Clontech). A few mussel protein products by other companies were also found (Adhesive Protein, Sigma Aldrich; MAP, Swedish Biosciences Laboratory; and AdheraCell, Genex Corp), but appear to be discontinued or out of business.

These products can either be cultured extractions of natural mussel adhesive proteins (MAPs) or synthetic proteins that mimic the natural materials. However, laboratory products have shown inferior strength compared to the natural protein. Most commercialized MAP sources are based on the marine blue mussel (*Mytilus edulis*) foot protein (Mefp), which has been designated Mefp-1. In 2007, the Idaho National Laboratory was conducting research on the large-scale production of recombinant Mefp-1 (Silverman et al. 2007). These efforts have since been abandoned. It was concluded that the exploration of mussel proteins as protective coatings for this project must also be abandoned, as it is cost-prohibitive for our experimental interests.

A review of the literature indicated that several significant advances in the understanding of the mussel adhesion mechanism occurred since the Bureau of Reclamation's (Reclamation) previous investigation (Skaja 2011). This report serves to update and document the state-of-the-art. Furthermore, Reclamation's researchers accepted an invitation to capture their knowledge as a chapter within the unpublished book entitled Biology & Management of Invasive Quagga/Zebra Mussels in the Western United States (Skaja et al. 2014, *in press*). Consequently, sections of this report have been forwarded for publication within that chapter.

INTRODUCTION

Invasive mussel species have received much attention since their introduction to United States freshwaters in the 1980s. The underwater surfaces of infested

waters are covered very quickly with the mussels, and there are no practical methods to mitigate their attachment. The organisms form small, adhesive bonds to these surfaces in a unique underwater cure mechanism. Researchers are very interested in understanding the processes of their distinctively strong, underwater attachment mechanism.

The motivations for researching mussel adhesives bridge several disciplines. For example, the biomedical industry is interested in utilizing MAPs for their inherent biocompatibility. The Biologically Inspired Materials Program within the National Institutes of Health has provided much support for academic research into mussel adhesion. To date, all commercial MAP products are marketed for the biomedical industry's use. However, materials scientists seek an opportunity to apply the mussel's unique adhesive properties to advance coating and adhesive sciences.

The numerous research efforts have created a wealth of information in scientific literature. Recent discoveries provide advanced theories in the mechanism of mussel plaque formation and its associated adhesive and cohesive chemistries.

Freshwater and Marine Mussel Species

Mussel species live throughout the world's freshwater and marine ecosystems. Many of these species are native. The zebra mussel (*Dreissena polymorpha*) is native to Eastern Europe. However, it was discovered in the Great Lakes in the early 1990s and significantly populated the eastern half of the United States in a very short period of time. Approximately 20 years later, the more versatile quagga mussel (*Dreissena bugenis*) began to out-compete the zebra populations where the two co-occur (Cohen, 2008). By 2007, the quagga mussel prevailed in the Eastern United States and had populated its first western waterway, the Colorado River. Both species area non-native to the United States and disturb the balance of native ecosystems largely due to a lack of predators.
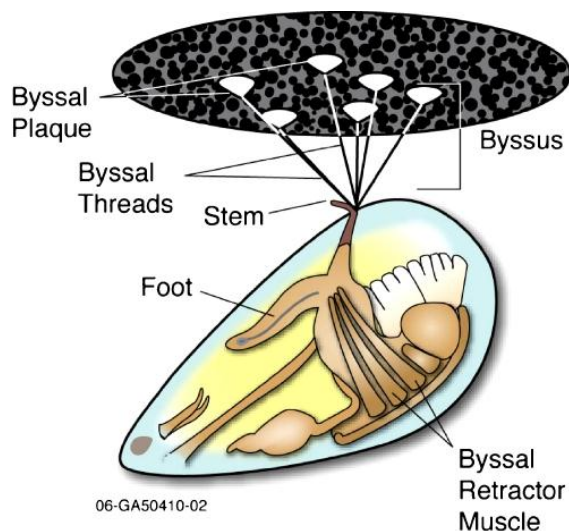
Many cities in the Western United States depend on the U.S. Department of the Interior, Bureau of Reclamation, to provide water and power. The uncontrolled colonies of mussels interrupt the operation of Reclamation water and power infrastructure by blocking or stopping flow to small-diameter pipes and intake equipment. The deceased animals cause similar havoc due to shell debris.

Mussel Attachment

This report reviews advancements in the understanding of how mussels produce the unique adhesion layer formed at the mussel plaque/substrate interface. By

revealing the mystery of their underwater adhesion, these theories can be applied to the development of new coatings, materials, or technologies that may significantly reduce or prevent the rate of attachment; which is needed to sustain the reliability of Reclamation operations. A basic overview is introduced here.

Mussel animals occupy the interior of their shell, while their exogenous byssus anchors them to an exterior surface. The byssus is produced by a foot organ. This foot reaches out from within the shell to probe the surrounding surfaces. Once a suitable substrate is selected, the tip of the foot is placed against it, which triggers the organism to initiate a series of adhesive protein secretions. The material cures very quickly, and the foot is lifted to reveal a single thread that connects the mussel and its shell to a small adhesive pad, or plaque, attached to the surface. The process takes approximately 5 minutes and is repeated to give the animal multiple anchor points. Hwang et al. (2013) provides an excellent, detailed description of the attachment process. Figure 1 highlights the anatomy of the mussel animal. Note that its foot and byssus (plaque and thread) are of interest for this review.

Figure 1.—Schematic of mussel animal attached to a substrate via byssus components. (Reprinted from Silverman and Roberto 2007)

The attachment process is similar throughout all mussel species. The number of foot proteins also appears to be common. However, the amino acid sequences and concentrations vary from species to species, which is likely a result of the species inhabiting different ecosystems in which water chemistry, predators, and environmental conditions are distinct.

MUSSEL PLAQUE COMPONENTS

Foot Proteins

The marine blue mussel (*Mytilus edulis*) has served as the industry standard for MAP research and development. A majority of the research is geared toward the biomaterial and medical engineering and commercialization of the mussel adhesive. Nevertheless, a basic theory of the adhesive protein's chemical composition and plaque formation has been gained along the way, and this knowledge is valuable to research and development for innovative materials such as underwater-cure coatings.

Nine proteins have been identified which participate in plaque formation: mussel foot protein (mfp)-1, 2, 3, 4, 5, and 6 as well as precollagen-D, -HG, and the thread matrix protein. The final three are found in the byssal thread. Some researchers suggest that there is a tenth protein; however, the widely variant mfp-3 may be the reason. The scope of this report investigates only those proteins contributing to the mussel plaque and primer layer since this is the source of the adhesion properties of interest.

Skaja (2011) describes the material properties for the foot proteins in a previous literature review. The report also details the physical and mechanical properties of the mussel plaque, adhesion strengths of several species, and a biochemical characterization highlighting the amino acid 3, 4-dihydroxy phenylalanine (Dopa) as a key component in the mussel adhesion mechanism. The section below highlights scientific advancements made in this area since that report. Background knowledge is included and summarized as needed.

Characterization of Adhesive and Cohesive Proteins

All mfps contain Dopa, which is key to the animal's wet adhesion mechanism (Yu et al. 2011b; Nicklisch and Waite 2012). Dopa forms adhesive and cohesive bonds through its reduced and oxidized (Dopa quinone) states, respectively. A brief schematic of protein-bound Dopa's diverse interactions is given on figure 2.

The left side of the figure demonstrates Dopa's adhesive roles. The oxidized Dopa can only lead to cohesive bonding. The one exception to this is if the quinone is reduced back to Dopa. This occurs through a thiol-containing reduction "partner protein," which will be described in the "Redox Chemistry" section (Yu et al. 2011a). An important note to stress here is that reduced Dopa leads to the strongest surface bonding (Lee et al. 2006). The reported routes for Dopa surface bonding at the byssal plaque interface include bidentate hydrogen
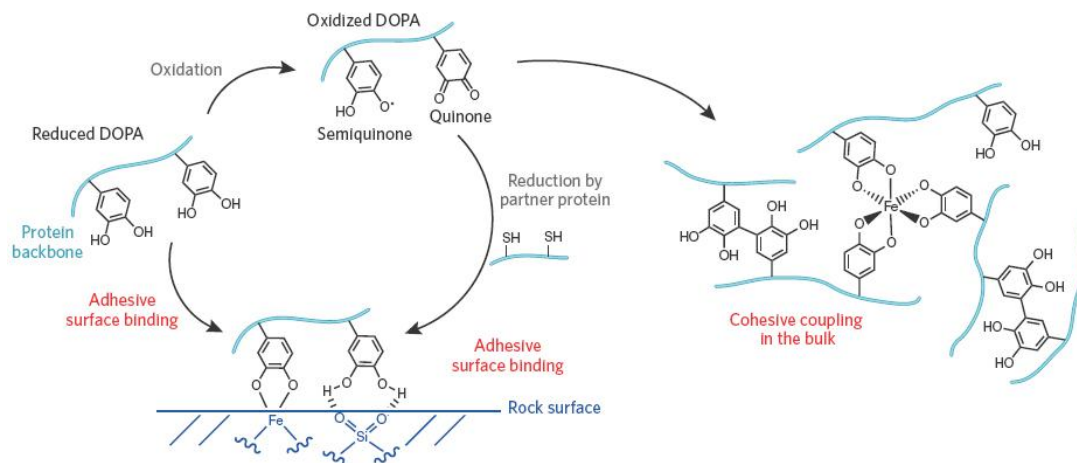
Figure 2.—Dopa forms strong adhesive bonds by bidentate hydrogen bonding and metal/metal oxide coordination (left). Dopa that has been oxidized to Dopa quinone can be reduced by thiol-containing groups to form adhesive bonds (center). Dopa quinone can also participate in cohesive oxidative crosslinking and metal chelation to form the bulk material (right).
(Reprinted from Wilker 2011)

bonding (Yu et al. 2011a), metal/metal oxide coordination (Lee et al. 2006), and oxidative cross-linking (Wilker 2011). Dopa has been known to chelate with metal ions such as calcium, iron, and aluminum (Brazee and Carrington 2006). Bidentate hydrogen bonding is twice as strong as a single hydrogen bond, making Dopa oxidation of this structure highly improbable (Yu et al. 2011a).

The right side of figure 2 demonstrates some of the cohesive bonds formed by Dopa or oxidized Dopa (quinones) in the byssal plaque or thread. Potential cohesive interactions include metal-mediated bonding, especially with iron, and intrinsic binding as well as oxidative covalent crosslinks (Nicklisch and Waite 2012). Cohesive bonds are vital to the development of the bulk material for the thread and plaque.

Each foot protein is composed of unique amino acid sequences and molecular weights. These vary slightly from species to species and ocean to freshwater. The percentage of Dopa contributes significantly to each protein's structural role. Table 1 summarizes these details as well as the molecular weights for the blue (Mefp-), zebra (Dpfp-), and quagga (Dbfp-) mussel proteins. Notice that Dpfp-4,-5,-6 and Dbfp-3,-4,-5,-6 have not yet been characterized. See references by Anderson and Waite (2002), Frank and Belfort (2002), Nicklisch and Waite (2012), Rzepecki and Waite (1993a, 1993b), and Silverman and Roberto (2007) for more information on these characterizations.

Table 1.—The location, molecular weight, and DOPA concentration are provided for blue, zebra, and quagga mussel foot proteins characterized to date.

Protein	Location (role)	Molecular weight (kDa)[1]	DOPA (percent)
Mefp-1	Thread and plaque outer layer (cohesion)	115	10–15
Mefp-2	Plaque matrix (25–40 percent of total matrix, cohesion)	47	2-3
Mefp-3	Plaque interface (adhesion)	5-7	20–25
Mefp-4	Plaque/thread junction (cohesion)	79	4
Mefp-5	Plaque interface (adhesion)	9.5	27
Mefp-6	Plaque matrix (cohesion)	11.5	Small
Dpfp-1	Thread and plaque outer layer (cohesion)	76	6.6
Dpfp-2	Plaque matrix (adhesion)	26	7
Dpfp-3	Plaque interface (adhesion)	4.5-7	N/A
Dbfp-1	Thread and plaque outer layer (cohesion)	76	0.6
Dbfp-2	Plaque matrix (cohesion)	35	2

[1] kDa = atomic mass unit, kilodalton

Organization of Foot Proteins

Figure 3 provides a generic schematic of the mfps and how they are organized across the interface, plaque, and thread. This figure is more representative of a marine mussel because the thread contains collagen (preCOL)—a combination of two proteins: precollagen-D and precollagen-HG—in addition to the thread matrix protein (tmp).
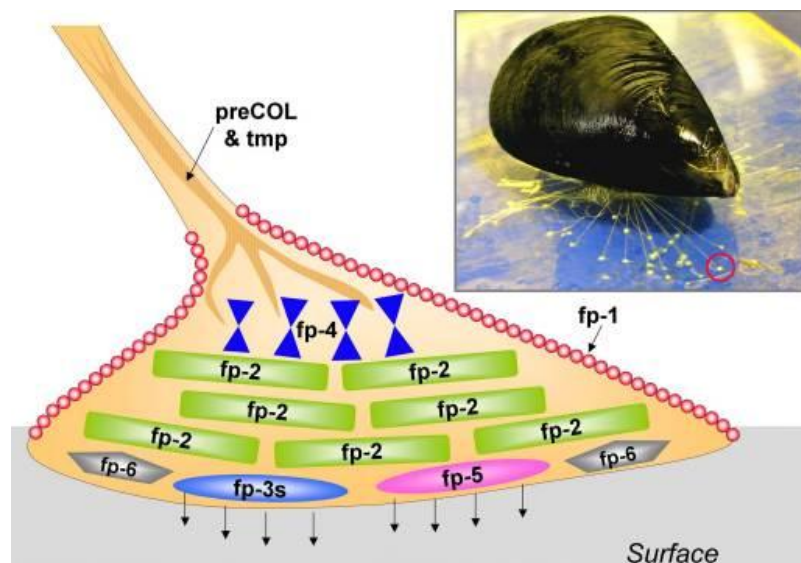

Figure 3.—Basic schematic of the byssal plaque and thread proteins. The organization of the foot proteins is thought to be similar for all mussel species. (Reprinted from Hwang et al. 2010)

MUSSEL ADHESION

Bonding Mechanisms

Physical adhesion between two materials is achieved through a combination of adsorption, mechanical interlocking, and molecular diffusion across an interface. It is vital to have intimate contact between the two materials. For byssal adhesion, microtopography, the viscosity of the adhesive, and wetting tendency are all important (Nalepa, and Schloesser 1993). The byssal adhesion mechanism of the zebra and quagga mussels is not completely understood, but mechanical interlocking is thought to play an important role. The mussel "interlocks" when the secreted adhesive flows into the microscopic pores and crevices of surfaces and cures. As stated previously, mfp-3 and -5 are very low molecular weight, which would allow better mobility for deep wetting and diffusion to occur.

Mussel adhesive also provides chemical functionality to form bidentate hydrogen bonding, chemical crosslinking, and covalent chelating bonding with metals (Nicklisch and Waite 2012). The foot proteins, mfp-3 and -5, also participate in the chemical adhesion to substrates. Here, dynamic amino acids, including Dopa, 4-hydroxyphenylalanine (tyrosine), and (S)-2-amino-3-(phosphonooxy) propionic acid (phosphoserine) seem to play significant roles by tightly bonding or chelating with substrate moieties.

It is most likely that a combination of mechanical and chemical adhesion mechanisms are utilized to form the characteristically strong byssal plaque/ substrate bonds. Further details on the byssal plaque formation, including this adhesive interface, are provided in the following sections.

Observations of Byssal Thread and Plaque Formation
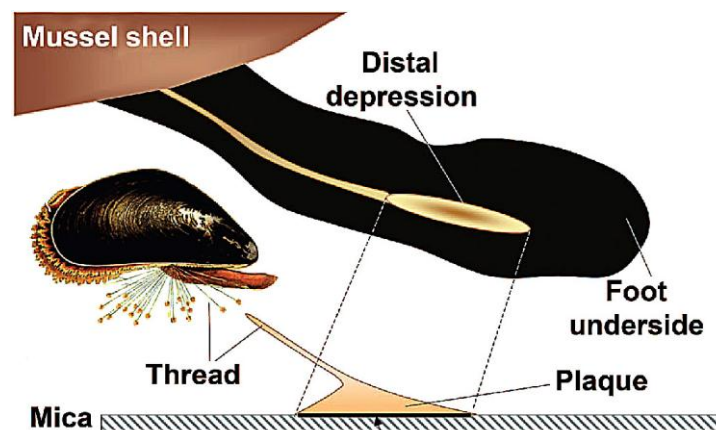
Figure 4.—The mussel foot is placed on the substrate for byssal formation within the distal depression.
(Reprinted from Hwang et al. 2010)

Direct observation of the zebra mussel byssus formation and zebra mussel attachment was conducted in 1990, and one interesting observation was that the mussel foot always swept across the substrate surface prior to attachment (Nalepa and Schloesser, 1993). Current theory suggests that the foot is used to clean the surface (Hwang et al. 2013). Once the surface is adequately prepared, the mussel foot becomes motionless as the byssus forms within the distal depression (Nicklisch and Waite 2012; Hwang et al. 2010). The distal depression is labeled within figure 4. The number of byssi formed depends water velocity, water temperature, salinity, food availability, etc., as well as substrate type (Rajagopal et al. 1996; Marsden and Lansky 2000).

The process of plaque formation in the blue mussel was recently investigated by real-time spectroscopic study of the protein secretions (Yu et al. 2011b). These studies included the natural, unperturbed adhesive secretion as well as chemically induced secretions. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry was the protein detection method used in the perturbed and unperturbed studies. Utilizing the previous biochemical characterizations allowed for a step-by-step analysis of the byssal plaque and thread formation.

Researchers achieved induced plaque formation by injecting 0.55 M potassium chloride (KCl) solution into the mussel's pedal ganglion organ at the base of the foot (Yu et al. 2011b). The plaque formation initiates within a minute of injection. Mefp-3 is the first detected protein, followed by Mefp-6 approximately 30 seconds later (Yu et al. 2011b). The MALDI-TOF also detects Mefp-5 during this first minute; however, this particular protein requires higher laser power for improved resolution. This is consistent with the organization of the proteins shown previously on figure 3.

The researchers used a number of quality control methods to compare the results of induced plaques to natural depositions. The secreted foot proteins, themselves, are identical; however, it is unknown whether the process of deposition is the same. The induced plaque formation experiments have been valuable in that they confirm and improve our understanding of each foot protein's role. It is assumed, here, that the zebra and quagga mussel byssal plaque and thread formation process is similar to that of the blue mussel.

CONDITIONS FOR PLAQUE FORMATION

Solution Chemistry

The water chemistry or solution conditions that occur under the foot during adhesive cure are vital to the formation of a strong, well-adhered plaque. Saltwater and freshwater are quite different, especially in regard to ionic strength.

Previously, it seemed that marine and freshwater mussels could have different attachment mechanisms due to different water chemistries. However, recent studies of the blue mussel discovered that the mussel foot isolates and facilitates a microenvironment during plaque formation. Yu et al. (2011b) utilized microelectrodes to reveal a pH of 5 and an ionic strength 0.1 M. The high viscosity and stickiness of the secreted proteins quickly fouls the electrode; therefore, these estimates are somewhat conservative (Yu et al. 2011b). This new information gives insight to the mussel's adhesive and cohesive processes.

Redox Chemistry

In addition to determining that the mussel foot was responsible for the pH and ionic strength regulation, the vital redox cycling processes were uncovered (Nicklisch and Waite 2012). The oxidation of Dopa to Dopa quinone results in reduced adhesion forces (Lee et al. 2006). The mussel carefully controls the redox chemistry of Dopa (and possibly other entities) to achieve proper plaque formation; too much oxidation leads to interfacial failure, and too little oxidation causes cohesive failure. Yet, more impressive is that the redox control is both spatial and temporal during byssal thread and plaque formation (Nicklisch and Waite 2012). The discovery of this insight occurred during research on marine mussels; however, it is believed that zebra and quagga mussels also utilize solution chemistry regulation and redox chemistries to achieve strong, covalent crosslinks.

Thiol-containing mfp-6 is responsible for balancing the redox chemistry in mussel adhesive and cohesive processes. The oxidation of Dopa to Dopa quinone is favorable over a wide range of pHs. The mfp-6 is co-secreted with mfp-3 and -5 to maintain a reducing environment for Dopa. Studies utilize the diphenylpicryl hydrazyl (DPPH) free radical to measure the redox species and their locations during plaque formation. The results confirmed the role of thiol-containing proteins, such as mfp-6, to reduce the Dopa lost to oxidation and metal chelation (Wilker 2011 Nicklisch and Waite 2012), which provides Dopa with added opportunities to form strong bidentate substrate bonds. Figure 5 demonstrates likely mfp-3/mfp-6 interaction pathways, including the reduction of Dopa quinone to form strong substrate bonds (at left) and mfp-6 secondary roles in forming thiol-dopa crosslinks with mfp-3 (at right) (Yu et al. 2011a).

METHODS FOR PREVENTING MUSSEL ATTACHMENT

It is believed that both zebra and quagga mussels contain high concentrations of Dopa in mfp-3 and -5 for adhesion, although the proteins have not been fully
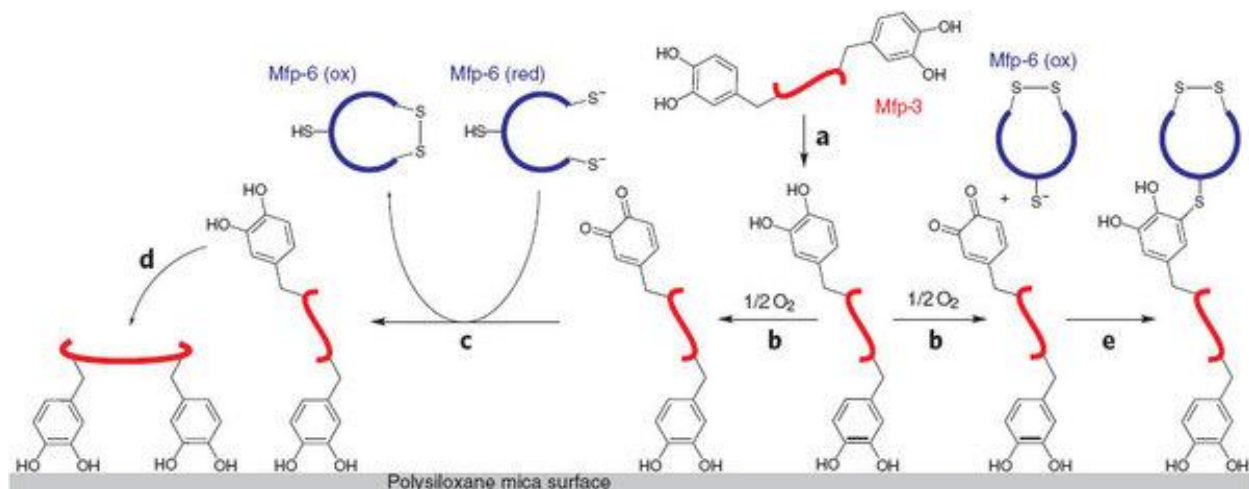
Figure 5. "(a–e) mfp-3 variants are secreted into the distal depression (a) and partially adsorbed by dopa mediated H-bonds to the mica surface. The oxidation of unadsorbed dopa to dopa-quinone (b) is counteracted by reducing thiolates (c) in mfp-6, which enables enhanced adsorption (d). Depletion of thiolate pairs in mfp-6 transforms mfp-6 into a crosslinker with mfp-3 (e) red, reduced, ox, oxidized."
(Reprinted from Yu et al. 2011a)

characterized. Dopa's primary interactions are strong, bidentate hydrogen bonding and covalent bonding with metals. Mussel adhesive adheres strongly to epoxy and polyurethane coatings (Skaja 2010). This adhesion is, in part, due to the oxygen and nitrogen chemistries within the coating that act as hydrogen bonding sites for the mussel adhesive (Skaja et al. 2013, *in press*). These functional groups are present at the surface in most organic coatings, allowing for facile mussel attachment. Coating chemistries can be designed to have no hydrogen bonding sites, such as polyethylene, polypropylene, polybutadiene, and polystyrene. For these systems, the ability for the mussel adhesive to wet and mechanically interlock with the surface microstructure and porosity becomes important. Thus far, the only nontoxic coating systems to prevent mussel attachment are the silicone foul-release coatings (Skaja 2010). This unique combination of chemistry and physical properties prevents mussel attachment; however, their durability may not be conducive to use on industrial equipment.

CONCLUSIONS

- Mussels form strong attachments to most natural and synthetic surfaces through an underwater curing of numerous byssal plaques and threads.

- The mussel foot is used to select and prepare a surface for attachment. The foot is then placed against the surface, and the process begins to build a plaque and thread. Within 5 minutes, the foot is lifted to reveal the thread/plaque. This process is repeated to form multiple attachments.

- The distal depression in the mussel foot is specially designed to form the mussel plaque. There are six foot proteins utilized in this process. Mfp-3 and -5 are adhesive and adhere to the substrate. Mfp-6 is cohesive and binds these proteins together. Mfp-2 is also cohesive and binds most of the plaque matrix together. Mfp-4 is cohesive and connects the plaque to the thread. Mfp-1 is cohesive and forms the plaque and thread outer sheath.

- The adhesion of the mussel plaque is achieved through both adhesion and mechanical interlocking. The amino acid protein 3,4-dihydroxyphenylalanine (Dopa) is key to the wet adhesion. The proteins form bidentate hydrogen bonding, metal/metal oxide coordination, and oxidative crosslinking. Mfp-3 and -5 have the highest concentration of Dopa of all foot proteins at 20–25 and 27 percent, respectively. They are also very low molecular weight, which likely causes them to have greater mobility to dissolve into the interstitial areas of a surface and bond by mechanical interlocking.

- Direct observation of mussel attachment was conducted in 1990. Recently, MALDI-TOF mass spectrometry was used to identify the foot proteins as they appear in byssal plaque and thread formation. The unique sequence of plaque formation begins with co-secretions of mfp-3 and -6. Mfp-5 is also detected during the first minute. Perturbed analyses were achieved by injecting 0.55 M KCl solution into the mussel's pedal ganglion organ at the base of the foot.

- The mussel foot isolates and facilitates a microenvironment during plaque formation. Microelectrodes revealed the pH and ionic strength to be ~5 and 0.1 M, respectively, at the initiation of the process.

- Mfp-6 is utilized by the mussel to control the redox cycling of various amino acid proteins. Dopa spontaneously oxidizes to Dopa quinone, which does not have adhesive bonding capabilities. The thiol-containing Mfp-6 reduces Dopa quinone back to Dopa to ensure strong adhesion.

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