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13. ABSTRACT (Maximum 200 words) The adhesive plaques of mussel (<i>Mytilus</i>) byssal threads leave behind residual prints when removed from various surface types. These prints are believed to contain the primary components responsible for opportunistic nonspecific mussels adhesion. Prints from all surfaces contain a 6 kDa dopa-rich peptide, mefp-3. In contrast, mefp-5 (9 kDa) can only be detected on steel. The high level of dopa in both proteins can be linked to cross-linking and chemisorption.				
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FINAL REPORT

GRANT #: NO0014-99-1-0774

PRINCIPAL INVESTIGATOR: J. Herbert Waite

INSTITUTIONS: University of California Santa Barbara

GRANT TITLE: Direct Analysis of Marine Interfaces: Mussels
and MALDI

AWARD PERIOD: 1 April 1999 to 1 April 2001

OBJECTIVES:

- 1- Identify and characterize interfacial proteins in the byssal attachment plaques of mussels (*Mytilus edulis*);
- 2- Identify the mode of post-translational modifications and chemical maturation in byssal proteins
- 3- Provide collaborative support for Flammang studies on the adhesive proteins in the Cuvierian tubes of holothuroids

APPROACH: 1- Interfacial proteins were identified by laser desorption mass spectrometry, protein characterization and cDNA sequencing. Byssal adhesive plaques were deposited by tethered mussels onto various surface types, removed from the surface using clean razor blades; adhesive faces were analyzed by MALDI TOF MS following preparation with matrix. Interfacial proteins were sequenced from the cDNA library of a single mussel using reverse transcriptase coupled to polymerase chain reaction.

2- Chemical maturation of byssal proteins was followed by MALDI TOF mass spectrometry and solid-state NMR especially cross-polarization magic angle spin (CPMAS) and rotational echo double resonance (REDOR). The ^{13}C and ^2H levels in analyzed byssus were enhanced by prior incorporation of labeled tyrosine analogues.

3- Collaborative support for the Cuvierian tubule studies underway at the University of Mons-Hainaut has involved primarily the following analyses: amino acid analysis of tube prints and electroblotted protein bands; Edman sequencing of Glu-C endoproteinase digests of tubule proteins, and MALDI TOF mass spectrometry.

ACCOMPLISHMENTS: 1. MALDI TOF mass spectrometry has been shown to be an indispensable tool for characterization of adhesive proteins. We have shown that mefp (*M. edulis* foot protein)-3 variants predominate near the interface between

plaque and substratum. Because the actual variants that are present differ extensively and given that MALDI TOF is capable of resolving many post-translational modifications, we have explored whether mussels tailor their deposition of mefps to surface-type. This directive follows the earlier observation that individual mussels contain mRNAs corresponding to at least 20 different Mefp-3s (Warner and Waite, 1999). We can now state with confidence that adhesive plaque formation by individual mussels on glass, stainless steel, and acrylic was consistent on all surfaces with regard to mefp-3 variants secreted (Floriolli, von Langen & Waite, 2000). Mefp-5, in contrast, appears to be specific to stainless steel surfaces. The role of post-translational modification in the variants with regard to surface type remains unclear. This question is complicated by the frequent m/z overlap of several variants exhibiting a spectrum of modification (hydroxylation and phosphorylation) ranging from all to none.

Breakthroughs were made in understanding the chemical maturation of byssal adhesive proteins (Burzio & Waite, 2000; McDowell et al., 1999). Solid-state NMR analysis of ^{13}C - and ^2H -enriched adhesive plaques was performed in collaboration with Prof. Jake Schaefer's lab in the Chemistry Dept at Washington University. Two hypotheses were tested: A. That chemical maturation of byssus in stationary seawater involves changes in the chemical coupling of aromatic residues such as tyrosine and dopa, and B. That maturation is linked to flow stress. *Hypothesis A* could not be sustained in stationary water systems. There was little detectable change in the reactivity of tyrosine or dopa. However, *B* showed a significant link between changes in aromatic chemistry and water flow. At flows of 20 cm/sec, NMR analyses revealed the formation of 5, 5'-didopa cross-links in adhesive plaques. These are the first cross-links to be identified in byssus. The production of didopa cross-links in oxidized mefps was confirmed using tyrosinase and/or periodate in vitro and followed by MALDI TOF mass spectrometry. Notably protein cross-linking could be competitively inhibited by addition of low molecular weight catechols.

CONCLUSIONS: 1) Individual mussels use the same suite of 4 to 6 mefp-3 variants when adhering to plastic, glass and steel. Mefp-5 was only observed on steel surfaces; 2) Proteins in byssal attachment plaques are cross-linked by formation of didopa. Cross-linking is enhanced by seawater flow and inhibited in vitro by addition of catechols; 3) The glue prints of *Holothuria forskalii* are acidic proteins

ranging in mass from 17 kDa to 200 kDa. Complete biochemical details are available in the ONR report submitted by Jangoux and Flammang.

SIGNIFICANCE: At least some of the fouling organisms (mussels, tubeworms) plaguing Navy ships have attachment strategies that rely on molecules that require chemical maturation. This is not unlike the curing reaction that is necessary for proper hardening in epoxy cements among others. We have shown for the first time that the cross-link formed in these natural thermosets is a didopa adduct. This has ramifications for replication of synthetic bioadhesives and the control of adhesion.

AWARD INFORMATION: None

PUBLICATIONS AND ABSTRACTS (1999-2001):

- McDowell, L. M., Burzio, L.A., Waite, J. H., and Schaefer, J. (1999) REDOR Detection of cross-links formed in mussel byssus under high flow stress. *J. Biol. Chem.* 274: 20293-20295.
- Waite, J.H. (1999). Reverse engineering of bioadhesion in marine mussels. *Ann. NY Acad. Sci.* 875: 301-309.
- Floriolli, R. Y. von Langen, J. and Waite, J. H. (2000). Marine surfaces and the expression of specific byssal adhesive protein variants in *Mytilus*. *Mar. Biotech* 2: 352-363.
- Anderson, K.E., and Waite, J.H. (2000). Immunolocalization of Dpfp1, a byssal protein of the zebra mussel (*Dreissena polymorpha*). *J. Exp. Biol.* 203: 3065-3076.
- Coyne, K.J. and Waite, J.H. (2000) In search of molecular dovetails in byssus: From the threads to the stem. *J. Exp. Biol* 203: 1425-1431.
- Burzio, L. A., Saez, C., Pardo, J., Waite, J.H. & Burzio, L. O. (2000) The adhesive protein of *Choromytilus chorus* (Molina 1782) and *Aulacomya ater*: a proline rich and a glycine-rich polyphenolic protein. *Biochim. Biophys. Acta* 1479: 315-320
- Burzio, L.A. and Waite, J. H. (2000) Cross-linking in adhesive quinoproteins: Studies with model decapeptides. *Biochemistry* 39: 111-47-11153.
- Harder, P., Grunze, M. and Waite, J.H. (2000) Interaction of the adhesive protein mefp-1 and fibrinogen with methyl and oligo(ethylene glycol) terminated self-assembled monolayers. *J. Adhesion* 73: 161-177.

Burzio, L.A. & Waite, J.H. (2001). Reactivity of peptidyl tyrosine by hydroxylation and cross-linking. *Protein Science* 10: 735-740.

Anderson, K. E. & Waite, J. H. (2001) Characterization of dbfp1 a byssal precursor of *Dreissena bugensis*. Biofouling. In press.