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September 18, 1997

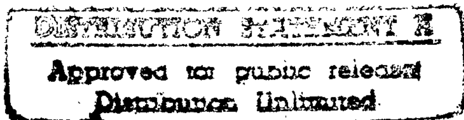
Dr. Keith B. Ward
Office of Naval Research
335/ONR/FR57
800 North Quincy Street,
Arlington, VA 22217-5660

Re: "Interfacial Culprits: Targetting Proteins of Byssal Adhesion"
Principal Investigator: Dr. J. Herbert Waite.

Dear Dr. Ward:

Enclosed is the final report for ONR grant N00014-89-J-3121. The report summarizes the significant results obtained during the entire period of support and is being distributed as per your recommendations. Please call if you have questions or need additional information.

Sincerely,



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J. Herbert Waite
Professor, Marine Chemistry/ Biochemistry

cc: Defense Technical Information Center (DTIC)
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Marine mussels (*Mytilus*) form permanent adhesive bonds with hard surfaces in their environment. The adhesive plaques of the byssus are situated in closest proximity with the bonded foreign surface. Of the four proteins known to be present in byssal plaques, we have isolated, characterized, and cloned several variants of Mefp-3. These are small basic proteins (5 to 7 kDa) with two prominent post-translational modifications, 3, 4-dihydroxyphenylalanine and 4-hydroxyarginine. Laser desorption studies of byssal plaques indicate Mefp-3s to occur at or near the interface.

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★ U.S.GPO:1991-0-296-362

FINAL REPORT

Grant#: N00014-89-J-3121

PRINCIPAL INVESTIGATOR: Dr. Herbert Waite

INSTITUTION: University of Delaware

GRANT TITLE: Interfacial Culprits: Targetting Proteins of Byssal Adhesion

AWARD PERIOD: 1 January 1993 - 30 September 1996

OBJECTIVE: To investigate the identity and distribution of proteins at or near the adhesive interface of the byssus, the holdfast structure in fouling marine mussels (*Mytilus*).

APPROACH: Normal cross-linking of proteins in the byssal adhesive plaques is perturbed by a temperature shock i.e. 18° to 8°C. Proteins are extracted from the plaques using acidic buffers containing 8M urea. These proteins are purified by reversed phase HPLC and sequenced by Edman chemistry and matrix-assisted laser desorption ionization mass spectrometry coupled with carboxypeptidase digestion. Sequence of other variants is generated by the polymerase chain reaction using oligonucleotide primers constructed from N- and C- terminal sequences.

ACCOMPLISHMENTS: Mefp-3 is a family of small proteins ranging in size from 5,000 to 6,500 daltons. All have similar compositions in which glycine, asparagine, 3,4-dihydroxyphenylalanine (DOPA) and tryptophan prevail, and all contain the exotic modification 4-hydroxyarginine. Of the estimated 30 or more variants in the family, we used pulsed liquid automated Edman sequencing in combination with matrix-assisted laser desorption ionization mass spectrometry (MALDI MS) to elucidate the primary structure of variant F (collaborator V. Papov at MIT). Using the sequence, we (collaborator K. Inoue at MBI, Japan) have constructed synthetic obligonucleotide primers to reverse transcribe and PCR-amplify related mRNA transcripts and derive complete sequences for these using PCR rapid amplification of cDNA ends (RACE). The complete cDNAs of seven variants have been discovered and many others are suggested. These results reflect a conserved N-terminus and hypervariable C-terminus. The functional basis for variability is not known. In order to determine whether Mefp-3 has a role in byssal adhesion, we (collaborator Mark Ross at NRL) have irradiated the underside of α-cyano 4-hydroxycinnamic acid-impregnated byssal plaques. It was found

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that molecules with masses in the range 5-6.5 kDa prevail near the interface. Many of these correspond to Mefp-3 precursors isolated from the foot, even to the point of having similar distributions in arginine hydroxylation. Curiously, there appears to be some correlation between surface type, e.g. glass, steel, polyethylene etc, and the variant of Mefp-3 detected.

Methods developed during the grant period include a temperature-induced retardation of cross-linking in the byssus, protocol for isolation of a highly basic family of peptides, MS·MS characterization of hydroxylated post-translational modifications, laser desorption of interfacial proteins from the adhesive plaques, and a technique for measuring the stability constants between DOPA containing peptides and Fe (III).

CONCLUSIONS: Marine mussels (*Mytilus* species) are one of many invertebrate groups that form permanent adhesive bonds with hard surfaces in their environment. The adhesive plaques of the byssus are situated in intimate proximity with the bonded foreign surface. We have isolated four proteins extracted from byssal plaques. Two were previously characterized: *Mytilus* foot proteins (Mefp) 1 and 2. A third, Mefp-3, has recently been sequenced by three independent methods: Edman degradation, laser desorption mass spectrometry, and cDNA sequences. Direct irradiation of matrix-impregnated plaque bases for laser desorption-ionization mass spectrometry suggests that Mefp-3 is located at or near the adhesive interface.

SIGNIFICANCE: Our studies suggest that Mefp-3 is at or near the adhesive interface of byssal plaques. Whether that makes it the culprit of fouling remains to be determined in future studies.

PATENT INFORMATION: None.

AWARD INFORMATION: None.

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