Role of Epicellular Molecules in the Selection of Particles by the Blue Mussel, *Mytilus edulis*

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Abstract. This study provides evidence that the suspension-feeding blue mussel, *Mytilus edulis*, uses biochemical cues to recognize its food. We identified lectins in mucus from the gills and labial palps, two pallial organs involved in the feeding process. These compounds were able to agglutinate rabbit and horse erythrocytes (RBC) and several species of marine microalgae representing different families. Additionally, the agglutination of RBC and microalgae was inhibited by several carbohydrates (fetuin, lipopolysaccharide (LPS), and mannose-related residues), suggesting that a suite of lectins may be present in mucus from the gills and labial palps. Results from feeding experiments, using microspheres with tailored surfaces, demonstrated that mussels preferentially ingested microspheres coated with the neoglycoproteins glucosamide-BSA and mannopyranosyl-phenyl-BSA but rejected in pseudofeces microspheres coated with BSA alone. The positive selection for neoglycoprotein-coated microspheres was inhibited when mussels were pre-incubated in seawater containing a solution of the same neoglycoprotein. Two surface properties of the microspheres, charge and wettability, had little effect on the observed selection process. Our results, along with our previous findings for oysters, suggest a new concept for the mechanism of particle selection in bivalves and perhaps other suspension-feeding organisms. Specifically, the selection of particles involves interactions between epiparticulate carbohydrates and lectins in the mucus produced by feeding organs.

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Introduction

In near-shore waters, suspension-feeding bivalves are confronted with a wide range of living and nonliving particles. Through several processes, bivalves are able to sort and ingest high-value particles in preference to low-value ones, thus enhancing the nutritive value of ingested material and optimizing energy gain (Allen, 1921; Fox, 1936; Shumway et al., 1985; Defossez and Daguzan, 1996; Pastoureaud et al., 1996; Ward et al., 1997, Ward and Shumway, 2004). The process by which particles are selected is not clear, and several possible mechanisms have been proposed to explain particle sorting, including the recognition of epiparticulate chemical compounds (see details in Ward and Shumway, 2004). Recently, it has been shown that particle processing in *Pecten maximus* (Beninger and Decottignies, 2005) and in *Crassostrea gigas* (Beninger et al., 2008) is directly related to the organic components present on the cell surface of diatoms. Additionally, Pales Espinosa et al. (2007) demonstrated that particle selection in the oyster *Crassostrea virginica* is mediated by extracellular metabolites associated with the cell surface of microalgae. Our latest investigations showed that particle sorting in this species results, at least partially, from interactions between carbohydrates associated with microalgal cell surface and lectins present in the mucus covering oyster feeding organs (Pales Espinosa et al., 2009, 2010). Common in many animal species including bivalves (Vasta and Marchalonis, 1982; Suzuki and Mori, 1989; Bulgakov et al., 2004; Tasumi and Vasta, 2007; Takahashi et al., 2008), lectins are a group of non-self recognition proteins that specifically and reversibly bind to sugar residues covering living cells (Sharon and Lis, 2004). Lectins have been shown or suspected to mediate symbiotic acquisition in corals (Wood-Charlson et al., 2006), nematodes (Bulgheresi et al., 2006), and the clam *Codakia or-
**Table 1**

Microalgal species used in the agglutination and feeding experiments.

<table>
<thead>
<tr>
<th>Species</th>
<th>Class</th>
<th>Ecological type</th>
<th>Size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isochrysis sp.</td>
<td>Prymnesiophyceae</td>
<td>planktonic</td>
<td>5–6</td>
</tr>
<tr>
<td>Nitzschia closterium</td>
<td>Diatom (pennate)</td>
<td>benthic/planktonic</td>
<td>17–20</td>
</tr>
<tr>
<td>Rhodomonas salina</td>
<td>Cryptophyceae</td>
<td>planktonic</td>
<td>15–18</td>
</tr>
<tr>
<td>Tetradesmis maculata</td>
<td>Prasinophyceae</td>
<td>planktonic</td>
<td>10–18</td>
</tr>
</tbody>
</table>

_bicul_ (Gourdin and Smith-Ravin, 2007). Furthermore, the involvement of lectins in the mechanism or mechanisms of prey selection by marine microorganisms was demonstrated by Wootton et al. (2007). These authors identified a mannose-binding lectin involved in trophic interactions between the predatory dinoflagellate _Oxyrrhis marina_ and _Isochrysis galbana_, its microalgal prey.

Nevertheless, the concept that particle sorting in bivalves is mediated by interactions between microagal cell-surface carbohydrates and lectins contained in pallial mucus is new and has been demonstrated only in the oyster _C. virginica_ (Pales Espinosa et al., 2009, 2010). The aim of the current study was to determine whether lectin involvement in particle selection is common across different bivalve taxa and study was to determine whether lectin involvement in particle selection is common across different bivalve taxa and...
Effect of 19 carbohydrates on hemagglutination activity of mucous extracts from the gills and labial palps of the mussel *Mytilus edulis*

### Table 2

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>Concentration</th>
<th>Mucus from gills</th>
<th>Mucus from labial palps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose-BSA</td>
<td>20 μg · ml⁻¹</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Curdlan</td>
<td>2.5 mg · ml⁻¹</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dextran</td>
<td>2.5 mg · ml⁻¹</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fucoid</td>
<td>2.5 mg · ml⁻¹</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Galactan</td>
<td>2.5 mg · ml⁻¹</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>36 mg · ml⁻¹</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose-BSA</td>
<td>20 μg · ml⁻¹</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>N-Acetyl-galactosamine</td>
<td>44.2 mg · ml⁻¹</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>N-Acetyl-glucosamine</td>
<td>44.2 mg · ml⁻¹</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucosamine-BSA</td>
<td>20 μg · ml⁻¹</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucose</td>
<td>36 mg · ml⁻¹</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>68 mg · ml⁻¹</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Laminarin</td>
<td>2.5 mg · ml⁻¹</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>2.5 mg · ml⁻¹</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Mannan</td>
<td>2.5 mg · ml⁻¹</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannopyranosylphenyl-BSA</td>
<td>20 μg · ml⁻¹</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannose</td>
<td>36 mg · ml⁻¹</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Mannose-BSA</td>
<td>20 μg · ml⁻¹</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Sucrose</td>
<td>68 mg · ml⁻¹</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Agglutination inhibition was tested with horse red blood cells. + = poor inhibition, ++ = moderate inhibition, +++ = strong inhibition; 0 = no inhibition.

Carbohydrates and mucous extracts from Mytilus edulis were added to 25-μl aliquots of plasma and mucous extract from *Mytilus edulis*. Thirty microliters of this suspension were added to wells containing 30 μl of mucous extracts. Each agglutination assay was performed in triplicate wells. After incubation (1 h at room temperature), plates were examined under an inverted microscope and agglutination levels in each well determined using a semiquantitative scale ranging from 0 (no agglutination) to 5 (strong agglutination) (adapted from Allen et al., 1977). Additionally, agglutination titer against RBC, which corresponds to the highest dilution factor that still yields hemagglutination, was determined using 2-fold serial dilutions.

To determine the carbohydrate specificity of lectins, hemagglutination-inhibition assays were performed using selected carbohydrates (Table 2; Sigma Aldrich, USA, and V-Labs, Inc., Covington, LA). Carbohydrates were added to mucous extracts from gills and labial palps, and incubated for 1 h at room temperature before RBC were added. Agglutination assays were then performed as described above.

### Selection of carbohydrate-coated microspheres

**Microsphere preparation.** Two types of carboxylated polystyrene microspheres (6-μm diameter, Bangs Laboratories, Inc., Fishers, IN), fluorescent dragon green (excitation/
emission = 480/520 nm) and fluorescent flash red (660/690 nm), were covalently coated with one of several compounds: bovine serum albumin (BSA; control) or one of two neoglycoproteins—glucosamide-BSA (Glu-BSA) or mannosylpyranosylphenyl-BSA (Mann-BSA). The PolyLink Protein Coupling kit (Bangs Laboratories) was used according to the manufacturer’s instructions. The two neoglycoproteins were selected for use in the feeding experiment because preliminary experiments showed that they inhibit the hemagglutination activity of lectins present in mussel mucus (see Results section). About 500 μg of BSA or one of the two neoglycoproteins were separately bound to 10^8 flash-red or dragon-green microspheres.

**Feeding experiments.** The general design followed our previously described protocols (Pales Espinosa et al., 2009). Equal numbers of microspheres with surface-coated BSA (fluorescent flash red) and neoglycoprotein (fluorescent dragon green; Glu-BSA or Mann-BSA) were suspended in filtered seawater (2 × 10^8 particles · ml^-1 total concentration). Mussels were placed in individual trays and delivered 1 liter of the microsphere suspension. Microspheres were kept suspended by gentle stirring. For each mussel, pseudo-1 liter of the microsphere suspension. Microspheres wereition). Mussels were placed in individual trays and delivered

Sorting inhibition experiments. To further elucidate the importance of lectin-neoglycoprotein interactions in particle selection, mussels were exposed to dissolved neoglycoproteins prior to and during feeding experiments. These treatments were included to determine if dissolved neoglycoproteins would interact with lectins associated with the feeding organs, thus reducing interactions with microsphere-coated neoglycoproteins. First, mussels were placed in individual trays filled with seawater containing 1 mg · 1^-1 of dissolved neoglycoproteins (Glu-BSA or Mann-BSA). After 30 min of incubation, each mussel was delivered a diet made of equal numbers of microspheres coated with either BSA or the neoglycoprotein that was also present in its feeding water (2 × 10^7 particles · ml^-1 total concentration). The procedures described above for feeding experiments were then followed. These data allowed us to determine if particle sorting by mussels could be inhibited by saturating mucus-associated lectins with neoglycoproteins.

**Surface properties of microspheres**

To examine the influence of surface properties on particle selection, charge and wettablility of the microspheres were determined. Microspheres with covalently attached BSA or neoglycoproteins (Glu-BSA or Mann-BSA) were prepared as described above. After being washed twice with Milli-Q water, a sample of each sphere type was incubated in filtered seawater (0.2 μm, salinity of 15, similar to that used in feeding assays) or in a solution of one of the two neoglycoproteins (1 mg · 1^-1 in filtered seawater, salinity of 15). Incubation with seawater or dissolved neoglycoproteins was done to mimic the conditions in the feeding and sorting-inhibition experiments, respectively.

The surface charge of microspheres was estimated on the basis of their electrophoretic mobility in filtered seawater at a salinity of 15 (pH 7.8). The surface charge of a particle in water is difficult to measure directly due to dissolved ions and adsorption of organic matter (Neihof and Loeb, 1974; Hunter and Liss, 1982). Instead, the sum of the electrostatic potentials around the particle, which is a function of the surface charge and termed the zeta potential, is determined (Abramson et al., 1942). For this measurement, microspheres were first incubated in 2-ml microcentrifuge tubes filled with 1 ml of one of the treatment solutions (as described above) for 30 to 60 min. After incubation, each suspension was transferred to a disposable capillary cell and analyzed for zeta potential using a Zetasizer-nano-ZS instrument (Malvern Instruments, Inc.). The accuracy and precision of this instrument is greatest when measurements are made in solutions with an ionic strength less than or equal to a salinity of 15. Standard operating procedures were used with a monomodal setting, a run temperature of 20 °C, and 19 sampling runs per determination. A new disposable capillary cell was used for each replicate determination because the high ionic conditions corroded the cell’s electrodes. Three replicate determinations were made for each of the different microsphere suspensions. Repli- cates were analyzed in an alternating fashion so that all suspensions had replicates that were incubated for 30 to 60 min. Zeta potential was calculated by the instrument using the Smoluchowski-equation setting.

The wettability of microspheres was determined by mea-suring the contact angle between a pad of the spheres and a drop of water in air (Hiemenz, 1986). Wettability is a function of hydrogen bonding and a rough indication of the surface free energy of the solid. In general, a hydrophilic surface produces a contact angle less than 90°, whereas a hydrophobic surface produces a contact angle of greater than 90° (Christenson, 1992; Good and van Oss, 1992). For this measurement, microspheres were first incubated for 30
min in 15-ml Falcon tubes filled with 5 ml of the appropriate solution. Tubes were then spun and supernatant removed. Ten milliliters of isotonic ammonium formate was added to displace salts (Pales Espinosa et al., 2009), and tubes were sonicated for 15 to 30 s in a bath sonicator to resuspend the microspheres. Suspensions of each sphere type and treatment were passed through individual 3-μm polycarbonate filters (2.5-cm diameter) to form a particle pad that completely covered the filter. Filters were then transferred to glass microscope slides and dried at 70 °C overnight. A 4-μl drop (Della Volpe et al., 2006) of Milli-Q water was placed on each pad and immediately photographed with a digital camera mounted on a horizontal dissecting scope (ca. 35× magnification). Contact angles were then calculated from digitized images using Image J software, ver. 1.38x (Abramoff et al., 2004), with the contact angle plugin. Two pads were prepared for each microsphere type and treatment, and a minimum of three replicate determinations were made. Blanks were also prepared by passing several milliliters of filtered seawater through polycarbonate filters and rinsing with ammonium formate. Surface roughness was assumed to be similar for all particle pads.

Data analysis

Data obtained from the feeding experiments were analyzed using goodness-of-fit tests (G test). Two series of tests were performed comparing the proportion of each type of microsphere in samples of the diet and biodeposits (pseudofeces, feces) collected from the mussels. The first series of tests ensured that, within each treatment, replicate samples of the diet, pseudofeces, and feces were homogeneous. The second series tested the null hypothesis that, within each treatment, the proportion of each microsphere type in the diet and the proportion of each in the pseudofeces or feces were not different. In addition to the comparison of raw counts, a sorting efficiency (SE) index was calculated in order to examine particle selection (Iglesias et al., 1992). This index was defined as

$$SE = 1 - \left( \frac{P}{D} \right),$$

where $P$ and $D$ represent the proportion of the particle of interest in the pseudofeces and diet, respectively. A positive SE for a given microsphere type indicates that it is preferentially ingested (i.e., lower proportion in the pseudofeces compared to the diet), a negative SE indicates rejection (i.e., higher proportion in the pseudofeces compared to the diet), and zero indicates the absence of selection. After confirming a normal distribution, calculated values of SE obtained for each of the two microsphere types in each treatment were compared to zero using a one-sample Student’s $t$-test (two-tailed). The null hypothesis was that the selection efficiencies were equal to zero (i.e., no selection). Two-sample $t$-tests were then used to examine differences in SE among mussels exposed or not exposed to dissolved neoglycoproteins.

Zeta-potential data for the three microsphere types (surface-coated BSA, Glu-BSA, or Mann-BSA) incubated in filtered seawater or a solution of one of two neoglycoproteins were analyzed using two-way analysis of variance procedures (ANOVA), followed by pairwise comparisons of means (Bonferroni). Sphere type and treatment were used as independent variables. Data were examined for normality and homoscedasticity prior to analysis. Contact angles were analyzed with a similar design, but procedures for circular-scale data were used (Watson-Williams $F$-test; Zar, 1984; Oriana Software, ver. 2.02, RockWare, Inc.). Unless otherwise indicated, all data are given as mean ± standard deviation (SD). For all statistical tests, a significance level of $\alpha = 0.05$ was used.

Results

Agglutination of microalgae by pallial-organ mucus

Protein concentration in plasma (557 ± 34 μg · ml⁻¹), and mucous extracts from gills and labial palps (1180 ± 87 and 335 ± 23 μg · ml⁻¹) were standardized to 300 μg · ml⁻¹ by dilution with ASW. Plasma and mucous extracts from Mytilus edulis agglutinated horse erythrocytes with comparable intensities (5.0 ± 0.3, 4.0 ± 0.1, and 4.5 ± 0.1, respectively), whereas no agglutination was found in the ASW control (Fig. 1). Both mucous extracts agglutinated three out of the four tested, taxonomically different, microalgal species (Fig. 1). Only Nitzschia closterium was poorly agglutinated by mucous extracts from gills (0.04 ± 0.2) and labial palps (0.12 ± 0.05). Agglutination levels ranged from 1.5 ± 0.5 for Isochrysis sp. incubated with gill mucous extracts to 5.0 ± 0.1 for Rhodomonas salina incubated with mucous extracts from the labial palps. It is noteworthy that the agglutination level was systematically higher for all algal species when tested with mucus collected from labial palps compared to gills. Further analysis using horse RBC demonstrated that the specific activity of lectins contained in labial-palp mucus from M. edulis was higher than the specific activity of lectins contained in gill mucus (Table 3).

Results of hemagglutination-inhibition experiments showed that the activity of lectins in mucous extracts from gills and labial palps was not inhibited by curdlan, dextran, or sucrose (Table 2). Hemagglutination activity of lectins was poorly inhibited by the presence of arabinose-BSA, galactan, galactose, N-acetylglucosamine, laminarin, and mannan. The highest inhibition of hemagglutination was obtained in the presence of lipopolysaccharide, fetuin, galactose-BSA, N-acetylgalactosamine, lactose, mannose, mannose-BSA, and to a lower level, glucosamine-BSA and manno-\alpha iranosylphenyl-BSA.
Selection of carbohydrate-coated microspheres

Two feeding experiments were conducted with mussels given BSA-coated microspheres (control) and microspheres coated with either (1) Glu-BSA or (2) Mann-BSA. In the first experiment, although the targeted proportion of each microsphere was 50% in the diet, an a posteriori count revealed a proportion of 39.4%/1.6% BSA-coated spheres to 60.6%/1.6% Glu-BSA-coated spheres in the suspension. In the second experiment, an a posteriori count revealed a proportion of 51.9%/1.9% BSA-coated spheres to 48.1%/1.9% Mann-BSA-coated spheres in the diet suspension. These proportions remained stable over the course of the experiment, indicating that differential settling of microspheres had not occurred.

In experiment 1, the proportion of BSA-coated microspheres significantly increased (n = 8, G test, P = 0.01) in the pseudofeces of mussels (47.8% ± 0.7%) compared to the diet, whereas the proportion of the Glu-BSA-coated spheres decreased (52.1% ± 0.7%). The trends in feces were opposite to those of the pseudofeces, with an increase in the proportion of Glu-BSA-coated microspheres (57.4% ± 5.4%) and a decrease in the proportion of BSA-coated spheres (42.6% ± 5.4%). Sorting efficiencies (SE) confirmed the above results. Mussels preferentially ingested Glu-BSA-coated microspheres but rejected BSA-coated spheres (Fig. 2, n = 8, t-test, P < 0.001). Results obtained in experiment 2 (i.e., BSA-coated and Mann-BSA-coated microspheres) were similar to those of experiment 1. Calculated values of SE showed that mussels preferentially ingested Mann-BSA-coated microspheres, while they rejected BSA-coated spheres (Fig. 2, n = 8, t-test, P < 0.001).

Table 3
Specific activity of lectins in mucous extracts from the gills and labial palps of the mussel Mytilus edulis (n = 3 pools of 20 mussels each) and the oyster Crassostrea virginica (n = 2 pools of 20 oysters each)

<table>
<thead>
<tr>
<th>Species</th>
<th>Pool #</th>
<th>Mucus origin</th>
<th>Total protein mg</th>
<th>Titer A.U.</th>
<th>Specific activity A.U./ml/mg</th>
<th>Palp/Gill specific activity ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. edulis</em></td>
<td>1</td>
<td>Gill</td>
<td>1.18</td>
<td>64,000</td>
<td>54,237</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palp</td>
<td>0.33</td>
<td>32,000</td>
<td>96,970</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Gill</td>
<td>0.93</td>
<td>32,000</td>
<td>34,411</td>
<td>2.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palp</td>
<td>0.19</td>
<td>16,000</td>
<td>85,879</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Gill</td>
<td>0.84</td>
<td>32,000</td>
<td>38,193</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palp</td>
<td>0.20</td>
<td>16,000</td>
<td>80,605</td>
<td></td>
</tr>
<tr>
<td><em>C. virginica</em></td>
<td>1</td>
<td>Gill</td>
<td>1.72</td>
<td>16,000</td>
<td>9,302</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palp</td>
<td>1.13</td>
<td>2,000</td>
<td>1,770</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Gill</td>
<td>0.98</td>
<td>16,000</td>
<td>16,343</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palp</td>
<td>0.81</td>
<td>4,000</td>
<td>4,975</td>
<td></td>
</tr>
</tbody>
</table>

Agglutination assays were performed using horse red blood cells.
Sorting inhibition experiments

Two sorting inhibition experiments were also conducted by exposing mussels to a mixture of two types of spheres (target proportion for the sphere types was 50/50). In the first, where mussels were exposed to a solution of Glu-BSA, an *a posteriori* count revealed a proportion of 41.1% ± 1.6% BSA-coated spheres to 58.9% ± 1.6% Glu-BSA-coated spheres in the diet suspension. In the second, where mussels were exposed to a solution of Mann-BSA, an *a posteriori* count revealed a proportion of 33.9% ± 2.7% BSA-coated spheres to 66.1% ± 2.7% Mann-BSA-coated spheres in diet. As in the selection experiments, these proportions remained stable over the course of the experiment, indicating that differential settling of microspheres had not occurred.

When mussels were exposed to dissolved Glu-BSA in seawater, the proportion of the two microsphere types in the pseudofeces and feces did not change compared to the diet (n = 7, G test, P > 0.05). Analysis of sorting efficiencies (SE) confirmed that mussels did not preferentially ingest or reject either microsphere type (Fig. 2, n = 7, t-test, P > 0.05). Similarly, when exposed to dissolved Mann-BSA, the proportion of the two microsphere types in the pseudofeces and feces did not change compared to the diet (n = 5, G test, P > 0.05). As with the first experiment, no particle selection occurred as SE values were not significantly different from zero (Fig. 2, n = 5, t-test, P > 0.05). It is noteworthy that the clearance rates of mussels exposed to the control (seawater) and to solutions of the neoglycoproteins were not significantly different (results not shown, t-test, P > 0.05).

Surface properties of microspheres

Experiments in which glucosamide was used as a treatment produced the following results. There was a significant effect of microsphere type (surface-coated BSA vs. Glu-BSA) and treatment (seawater vs. solution of Glu-BSA) on zeta potential (Table 4A, n = 12, two-way ANOVA, P < 0.01). Microsphere type also had a significant effect on contact angle, but treatment did not (n = 16, two-way Watson-Williams F, P < 0.01 and P > 0.05, respectively). Experiments in which mannopyranosylphenyl was used as a treatment produced the following results. Microsphere type (surface-coated BSA vs. Mann-BSA) had a significant effect on zeta potential, but treatment (seawater vs. solution of Mann-BSA) did not (Table 4B, n = 12, two-way ANOVA, P < 0.01 and P > 0.05, respectively). Microsphere type and treatment also had significant effects on contact angle (n = 16, two-way Watson-Williams F, P < 0.01). Importantly, in all cases there was a significant difference in zeta potential and contact angle between BSA-coated and neoglycoprotein-coated microspheres, regardless of treatment (Bonferroni and Watson-Williams pairwise comparisons, respec-
MECHANISM OF PARTICLE SELECTION IN THE BLUE MUSSEL

Table 4
Surface properties of microspheres (6 µm) with different coatings and treated with seawater or one of two neoglycoprotein solutions

<table>
<thead>
<tr>
<th>Microsphere Type</th>
<th>Zeta Potential (mV)</th>
<th>Contact Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seawater</td>
<td>Sol Glu-BSA</td>
</tr>
<tr>
<td>BSA-coated</td>
<td>−8.8 (1.0) a</td>
<td>−6.2 (0.6) b</td>
</tr>
<tr>
<td>Glu-BSA-coated</td>
<td>−4.6 (0.8) b, c</td>
<td>−3.8 (0.6) c</td>
</tr>
<tr>
<td>B.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zeta Potential (mV)</td>
<td>Contact Angle (°)</td>
</tr>
<tr>
<td></td>
<td>Seawater</td>
<td>Sol Mann-BSA</td>
</tr>
<tr>
<td>BSA-coated</td>
<td>−8.8 (1.0) a</td>
<td>−7.8 (0.9) a</td>
</tr>
<tr>
<td>Mann-BSA-coated</td>
<td>−4.6 (0.5) b</td>
<td>−5.0 (0.6) b</td>
</tr>
</tbody>
</table>

Note: Zeta potentials are presented as means of three replicate measurements ± standard deviation in parentheses. Contact angle data are given as mean vector (µ) of 4 to 5 replicate measurements ± circular standard deviation in parentheses. Within each of the four data sets, means with the same letters are not significantly different at the α = 0.05 level (Bonferroni pairwise comparisons).

A. Surface properties of microspheres (6 µm) coated with either bovine serum albumin (BSA) or glucosamide-BSA (Glu-BSA), and treated with either filtered seawater (0.2 µm) or a solution (1 mg·l⁻¹) of glucosamide-BSA (Sol Glu-BSA).

B. Surface properties of microspheres (6 µm) coated with either bovine serum albumin (BSA) or mannopyranosylphenyl-BSA (Mann-BSA), and treated with either filtered seawater (0.2 µm), or a solution (1 mg·l⁻¹) of mannopyranosylphenyl-BSA (Sol Mann-BSA).

Discussion

Although several studies have previously described the ability of suspension-feeding bivalves to select food particles prior to ingestion and reject undesirable material as pseudofeces (Pastoureaud et al., 1996; Ward et al., 1998; Ward and Shumway, 2004), the mechanisms of this process have remained unclear. Recently, we proposed that particle selection in the oyster Crassostrea virginica is at least partially mediated by interactions between lectins contained in mucus covering feeding organs and carbohydrates present at the surface of food particles (Pales Espinosa et al., 2009). The present study adds additional data to support our working hypothesis that carbohydrate-lectin interactions are important for particle recognition in suspension-feeding bivalves. In particular, we have demonstrated that such interactions can mediate particle selection in Mytilus edulis, the blue mussel, a bivalve with a different gill architecture and strategy for particle sorting than that of the oyster.

Lectins are a group of carbohydrate-binding proteins that specifically and reversibly bind to sugar molecules covering living cells (Sharon and Lis, 2004). These molecules are well known to play an important role in the recognition of foreign particles and, more specifically, in the defense system of vertebrates and invertebrates. Additionally, lectins are now recognized to be important in diverse processes in marine organisms that involve interactions between microalgae and invertebrates, such as symbiosis (Jimbo et al., 2000; Bulgheresi et al., 2006; Wood-Charlson et al., 2006; Kvennefors et al., 2008) and feeding processes (Wootton et al., 2007; Pales Espinosa et al., 2009). In the current study, we have shown that mucus covering the feeding organs of M. edulis contains lectins with diverse activity. Several carbohydrates with different structures were able to partially inhibit agglutination, suggesting the presence of a suite of lectins in mucus both from gills and from labial palps. In species of the Mytilidae, lectins have been identified in extracts from the whole animal (Belogortseva et al., 1998), in hemolymph (Tunkijanjuk et al., 1997; Barracco et al., 1999; Jayaraj et al., 2008), and in association with gametes (Takagi et al., 1994; Springer and Crespi, 2007). Additionally, the development of expressed-sequence-tag libraries has allowed for the identification of several specific lectins from M. edulis (Tanguy et al., 2008). Furthermore, the sialic-acid-binding lectin from Modiolus modiolus, the horse mussel (Tunkijanjuk et al., 1997), and the β-glucan-binding protein from Perna viridis, the green mussel (Jayaraj et al., 2008), are able to agglutinate bacteria and are thought to be involved in the defense mechanism in these species. Lysin-M7, which is thought to be a member of the C-type lectin family (Springer and Crespi, 2007), has been hypothesized to mediate sperm-egg interaction in Mytilus.
**galloprovincialis.** Our study is the first to report the presence of lectins in mucus covering the pallial organs of the mussel *M. edulis*. Particle selection has been described in several mussel species (Ward et al., 1998; Ke and Wang, 2002; Velasco and Navarro, 2002; Pales Espinosa et al., 2008) including *M. edulis* (Kiorboe et al., 1980; Cucci et al., 1985; Newell et al., 1989; Ward and Targett, 1989; Bougrier et al., 1997; Defossez and Hawkins, 1997). Our results indicate that the sorting mechanism of *M. edulis* is mediated by recognition molecules on the surfaces of particles. For instance, when mussels were fed microspheres coated with carbohydrates known to inhibit hemagglutination activity (e.g., Glu-BSA or Mann-BSA), a significantly greater number of these spheres were ingested compared to microspheres coated only with BSA, which were rejected in pseudofeces. More interestingly, the pre-incubation of mussels in seawater containing the dissolved neoglycoproteins inhibited selection of microspheres with the same surface-coated carbohydrate. The dissolved carbohydrates probably interacted with lectins contained in the mucus of the gills and labial palps, reducing subsequent interactions with carbohydrates bound to the microspheres and resulting in loss of particle-sorting capabilities. These results suggest that particle selection in mussels relies on a recognition mechanism involving lectins that interact with glucose and mannose residues.

Microspheres coated with the neoglycoproteins had significantly lower zeta potentials and contact angles than the same microspheres coated with BSA alone. Although treating these microspheres with a solution of neoglycoprotein significantly reduced zeta potential (i.e., BSA- and Glu-BSA-coated microspheres treated with a solution of Glu-BSA), or significantly increased contact angle (i.e., BSA- and Mann-BSA-coated microsphere treated with a solution of Mann-BSA), the significant differences between the two microsphere types remained (Table 4). These data suggest that surface charge and wettability had little effect on particle selection in our experiments. Mussels preferentially ingested microspheres coated with neoglycoproteins when exposed only to seawater, but demonstrated no selection when exposed to a solution of neoglycoprotein in seawater, even though under both treatments the surface characteristics of the two microsphere types were different.

Lectin-specific activity in mussels was noticeably higher in mucous extracts from the labial palps than in extracts from the gill (Table 3). The opposite trend was observed in the oyster *Crassostrea virginica*, which demonstrated higher specific activities in mucous extracts from the gill (Table 3). This dissimilarity may reflect differences in the functional morphology of the feeding organs and locus of particle selection. *Mytilus edulis* possesses a filibranch, homorhabdic gill composed of a single filament type, whereas *C. virginica* possesses a pseudolamellibranch, heterorhabdic gill composed of two different filament types. Results of previous studies indicate that the heterorhabdic gill of several oyster species (i.e., *Crassostrea virginica* and *C. gigas*) is capable of particle selection. In mussels (i.e., *M. edulis*, *M. trossulus*), however, the homorhabdic gills function little in particle sorting, and the labial palps are the main site of selection (Ward et al., 1997, 1998; Levinton et al., 2002). Therefore, the importance of each organ in the selection process of mussels and oysters matches well with their specific lectin activity. It is possible that the degree of involvement of each organ in particle selection is reflected in its lectin content, but this assessment remains highly speculative without further investigation.

Results obtained here for the blue mussel complement our prior work for the eastern oyster (Pales Espinosa et al., 2009), and provide additional evidence that particle sorting in suspension-feeding bivalves involves interactions between epiparticulate/cellular carbohydrates and lectins present in mucus covering the feeding organs. The identification and isolation of mucosal lectins would allow, at a molecular level, for a precise exploration of the mode of operation of these sugar-binding proteins in particle selection.

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**Literature Cited**


Beninger, P. G., A. Valdizan, B. Cognie, F. Guheneuf, and P. Decottignies. 2008. Wanted: alive and not dead: functioning diatom status...
is a quality cue for the suspension-feeder *Crassostrea gigas*. *J. Plankton Res.* **30**: 689–697.


Pales Espinosa, E., M. Perrigault, J. E. Ward, S. E. Shumway, and B. Allam. 2010. Microagal cell surface carbohydrates as recognition


History of lectins: from hemagglutinins to biological recognition molecules. *Glycobiology* 14: R53–R62R.


A galectin of unique domain organization from hemocytes of the eastern oyster (*Crassostrea virginica*) is a receptor for the protistan parasite *Perkinsus marinus*. *J. Immunol.* 179: 3086–3098.


*Biosstatistical Analysis.* Prentice-Hall, Englewood Cliffs, NJ.