Do outer membrane proteins of biofouling bacteria *Vibrio alginolyticus* have lectins?

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Received 18 January 2001; revised 15 November 2001

The outer membrane proteins (OMPs) of *Vibrio alginolyticus*, isolated from tin panels lowered into the seawater when subjected to SDS-PAGE analysis revealed proteins of molecular weight ranging from 14 – 116 kDa. However, when stained with PAS only 9 glycoprotein bands were observed. Sugar binding studies and hemagglutination assay performed with RBCs from various animals confirmed the absence of lectins. Apparently adhesion of *V. alginolyticus* to tin panels involves other interactions such as Van der Wall’s interaction and electrostatic forces.

**[Key words]:** Outer membrane, proteins, biofouling, *Vibrio alginolyticus*, lectins

Attachment of bacteria to the submerged structures in the sea is a complex phenomenon mediated by the production of adhesive exopolysaccharides, outer membrane proteins (OMPs), fimbriae and flagella and lipopolysaccharides. The bacteria that remain embedded in the glycoalyces besides serving as food for macrofouling invertebrate larvae also provide specific biochemical and molecular markers, which induce adhesion and metamorphosis of the larvae. The fouling process has obvious effects on the efficiency of movement of ships through water, the transfer of heat and the flow of water through fishing nets. The outer membrane proteins, mainly glycoproteins, have been implicated to mediate the recognition and adhesion process. The role of lectins as adhesins to biomaterials is well documented. The present investigation was undertaken to find out whether the outer membrane proteins of *Vibrio alginolyticus*, a marine biofoulant, contain lectins that serve in irreversible adherence to metal surfaces lowered into the sea.

A modified method of Kabir was used for isolation of OMPs from *Vibrio alginolyticus* (Division: Gracilicutes, Order: Enterobacteriales Family: Vibrionaceae). The cells grown in seawater nutrient (SWN) broth were harvested by centrifugation at 8000 x g for 15 min at 4°C and washed thrice with tris-HCl (50 mM, pH 7.5). Cells were suspended in tris buffer and lysed by sonication at 100 watts on ice for 2 min. This was repeated thrice with 2 min interval. Unbroken cells were removed by centrifugation at 5000 x g for 15 min at 4°C and supernatant retained. Total membranes were collected by centrifuging the supernatant at 50000 x g for 30 min at 4°C and resuspended in tris-HCl 50 mM, pH 7.5, containing 2% sodium lauryl sarcosinate and incubated at room temperature for 30 min. The pellet containing OMPs were collected by centrifugation at 50000 x g for 30 min at 4°C, suspended in sterile distilled water (glass distilled water sterilised in autoclave at 121°C for 15 min) and aliquots stored at –20°C until further use. Sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS–PAGE) was essentially according to the method of Laemmli. Proteins were stained by coomassie blue staining and glycoproteins were stained with periodic acid Schiff’s base staining (PAS). The method suggested by Gershoni was followed to determine whether the sugars inhibited the binding of OMPs to horse radish peroxidase (HRP). OMPs were coated onto a 96-well polystyrene plate, overnight. Unbound OMPs were washed off using tris-buffered saline (20 mM tris-HCl, pH 7.5, 0.15% NaCl). Polystyrene plates coated with OMPs were preincubated with sugar solutions (glucose, galactose, glucosamine, xanthan and EPS obtained from *V. alginolyticus*) at 50 mM concentration and the unbound sugars were washed off before HRP was added. After removal of excess HRP by washing with TBS, tetra
methyl benzidine (TMB) or hydrogen peroxide (H₂O₂) was added and absorbance measured at 490 nm. Concanavalin A (Con A) served as a control.

Hemagglutination was performed in the presence of OMPs using washed red blood cells (RBCs) of various animals viz., chicken, mice, rat, duck, rabbit and goat to test for the presence of lectins. RBCs of the animals were collected in Alsevers solution and washed with phosphate buffered saline (100 mM, pH 7.4, 0.9% sodium chloride). To 96-well round bottom polystyrene plates, OMPs (200 μg/ml) were added and serially diluted. After addition of washed RBCs (100 μl) in each well, the plates were incubated at 37°C for 1 h. Con A was used as a positive control.

The adhesion of marine bacteria to metal panels lowered in the sea is analogous to receptor-ligand interactions observed in human and plant pathogenic bacteria. In order to find out whether the V. alginolyticus isolated from tin panels lowered into the sea contained any glycoproteins, the OMPs were isolated and run on SDS-PAGE. A protein molecular weight distribution ranging from 14-116 kDa was seen on staining with coomassie blue. However, when stained with PAS only 9 glycoprotein bands were observed.

Table 1 — Interaction between various sugars and OMPs bound to microtiter plates

<table>
<thead>
<tr>
<th>Sugars</th>
<th>% increase in binding of HRP to OMPs*</th>
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<tbody>
<tr>
<td>Glucose</td>
<td>36.92 ± 2.2</td>
</tr>
<tr>
<td>Galactose</td>
<td>42.65 ± 3.2</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>10.75 ± 6.8</td>
</tr>
<tr>
<td>Xanthan</td>
<td>2.51 ± 6.5</td>
</tr>
<tr>
<td>EPS (V. alginolyticus)</td>
<td>4.30 ± 7.2</td>
</tr>
</tbody>
</table>

Microtitre plates coated with OMPs (50μg/well) were preincubated with sugars (50 mM) for 1 hr at 37°C and unbound sugars were washed off before HRP was added. The bound HRP was estimated as per the method of Gershoni (1988). Values presented are the mean ± SD of triplicate determinations of each representative experiment. Control (no sugar) was normalized to 100.

Table 2 — Hemagglutination assay of OMPs using various animal RBCs

<table>
<thead>
<tr>
<th>RBCs of Different Animals</th>
<th>OMP (200 μg/ml)</th>
<th>Con A (10 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>Negative</td>
<td>256 HA units</td>
</tr>
<tr>
<td>Mice</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Rat</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Duck</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Goat</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

OMPs (200 μg/ml) from V. alginolyticus were serially diluted in round bottomed polystyrene plates, to which PBS washed RBCs (100 μl) of different animals were added. Positive = mat formation, Negative = bottom formation.

(Fig. 1). To confirm whether any of the glycoproteins obtained from OMPs of V. alginolyticus was a lectin, sugar binding studies were carried out. The rationale used in this study was that when the sugar remains bound to OMPs, the HRP would not be expected to bind. Consequently a decrease in absorbance values should result. In the present study, the increase in percent absorbance in the presence of different sugars (Table 1), galactose giving the maximum (42.65%) proved that the OMPs from V. alginolyticus did not contain lectins.

In order to further confirm that the OMPs did not contain lectins, the isolated OMPs were subjected to hemeagglutination assay with washed RBCs from different animals, with Con A as the control. The results in Table 2 clearly revealed that hemeagglutination assay was positive only in case of Con A with chicken RBCs. No hemeagglutination was observed with any of the RBCs in the presence of OMPs at as
high a concentration of 200 μg/ml. Jansen et al.\textsuperscript{15} employing hemeagglutination assay have identified surface lectins on \textit{Staphyloccocus saprophyticus} S1 and \textit{Pseudomonas aeruginosa} (ATCC 27853) that showed specificity to N-acetylglactosamine/N-acetylglucosamine and N-acetylneuramic acid, respectively. In \textit{Hyphomonas} strain MHS-3, the EPS has been observed to show specificity to N-acetyl-D-galactosamine\textsuperscript{4}. In the present study since the OMPs do not contain lectins, apparently adhesion of \textit{V. alginolyticus} to tin panels involves other interactions such as van der Waal’s interaction and electrostatic forces. Busscher et al.\textsuperscript{10} have reported that while van der Waal’s interactions occur between all adjacent phases regardless of composition and act over relatively long separation distances (>50 nm), electrostatic potential becomes significantly involved at closer separation distances (10-20 nm) and could be attractive or repulsive between the two surfaces. However, Weincek\textsuperscript{17} reported that repulsion forces decrease with an increase in ionic strength of the medium and many natural environments such as seawater, have sufficient electrolyte concentrations to eliminate the electrostatic repulsion barrier.

References