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MANNOSE ADHESIN–GLYCAN INTERACTIONS IN THE *EUPRYMNA SCOLOPES–VIBRIO FISCHERI* SYMBIOSIS

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The recognition of and adherence to animal epithelia by bacteria is often mediated by bacterial surface lectins, called adhesins, that bind to sugars on the brush borders of host cells (Giampapa *et al.*, 1987; Abraham *et al.*, 1988; Sharon *et al.*, 1989). We report here results of experiments that provide evidence for the role of adhesin-glycan interactions in the initiation of the model symbiosis (Ruby, 1996) between the sepiolid squid *Euprymna scolopes* and its light organ symbiont *Vibrio fischeri*.

1. MATERIALS AND METHODS

Animals were collected and maintained as previously described (McFall-Ngai *et al.*, 1991). To investigate whether bacterial adhesin-animal glycan interactions are involved in the successful colonization of the *E. scolopes* light organ by *V. fischeri*, we determined: i) the type of adhesin(s) present on the surfaces of *V. fischeri*; ii) the nature of the glycans on light organ epithelia that interface with the symbionts; and, iii) the ability of sugars and sugar analogs to inhibit the experimental inoculation of the symbiotic organ.

Standard hemagglutination assays were employed for the detection of adhesins on *V. fischeri* (Duguid *et al.*, 1980). A hemagglutination deficient mutant of *E. coli* (pilE$^-$, strain

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ORN133) and its parent strain (Maurer *et al.*, 1987) were used as negative and positive controls, respectively. Sugars on the surfaces of light organ host cells were detected by light microscopy on paraffin-embedded tissue sections using specific lectins conjugated to colorimetric reporters (Bry *et al.*, 1996). In colonization experiments, a light organ isolate, *V. fischeri* strain ES114 (Lee *et al.*, 1994), was grown in seawater-based media, washed and inoculated into seawater containing sugars or sugar analogs; specific sugars that are metabolizable by *V. fischeri* were substituted with nonmetabolizable analogs. After 15 min, the seawater was dispensed as 5 ml aliquots into scintillation vials and a single newly hatched squid was introduced to each vial. Host tissue colonization was detected both by monitoring *V. fischeri* luminescence with a photometer and by enumerating colony-forming units (CFU) of *V. fischeri* that resulted from the plating of light organ homogenates.

2. RESULTS

In analyses of *V. fischeri* for the presence of specific adhesins, both the symbiosis-competent strain ES114 and the symbiosis-noncompetent strain MJ1 were found to agglutinate guinea pig red blood cells (RBCs). These data suggest that *V. fischeri* has mannose adhesins either on fimbriae or on the cell wall.

Because these results suggested that *V. fischeri* has mannose-recognizing adhesins on their surfaces, we analyzed host tissues for the presence of mannose residues that might behave as receptors for these adhesins. Histological sections of the juvenile light organ stained positively for the presence of mannose along the apical surfaces of the host epithelial cells, whereas there was no detectable staining in samples incubated with the fucose-recognizing lectin, which was used as a control for the possible non-specific binding of lectins to host tissue. In addition, we analyzed host tissue extracts for the presence of 5 common glycans (Glycan Differentiation Kit, Boehringer Mannheim, Inc., Indianapolis, IN, USA). Whereas control extracts from mantle tissue had all 5 of the glycans detected by this method, only mannose was abundant enough in light organ extracts to be detected.

In determinations of the ability of several sugars and sugar analogs to block infection (Fig. 1), only the mannose analog, methyl-a-D-mannopyranoside (Sigma Chemical Co., St. Louis, MO, USA), was capable of blocking infection; i.e., only the animals exposed to this sugar under prescribed conditions (see below) showed neither luminescence nor detectable CFUs in light organ homogenates. In the presence of other sugars or sugar

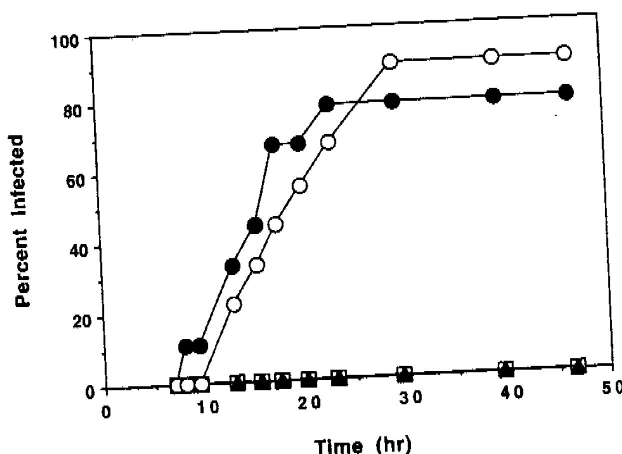

Figure 1. The effect of the presence of sugars on colonization of the *E. scolopes* light organ. Exposure to sugars (0.25%) such as a galactose analog (○), fucose, or a glucose analog resulted in similar colonization levels of the light organ as control animals not exposed to sugars during the colonization period (●). Exposure to the mannose analog (0.25%) inhibited infection fully (□). The control animals not exposed to added *V. fischeri* (▲) also showed no light organ colonization.

analogs, under all conditions, the juveniles exhibited luminescence and averaged 3.0×10^5 CFU/animal, similar to the colonization level of control animals not exposed to sugars. The growth conditions of the bacteria, i.e., grown in either a rich or a minimal medium, had no discernible effect on the inhibition patterns of the sugars.

We investigated the conditions under which mannose analogs were capable of inhibiting inoculation of the light organ. With strain ES114 at a concentration of 700 cells/ml, the mannose analog at concentrations from 2% down to 0.25% was capable of blocking infection in 100% of the juveniles. [Animals were healthy for up to 6 d when continuously exposed to 2% sugar in seawater; however, at higher percentages of sugar, they showed signs of stress, and 5% sugar was lethal within a few hours.] At slightly higher inoculation densities of the bacteria, approximately 1000 cells/ml, the mannose analogs were less effective at blocking infection. However, in those animals that did become infected, the onset of infection was always delayed by several hours relative to control animals, and the extent of the infection was always lower in comparison with controls exposed to no sugar or sugars other than the mannose analog. When bacteria were present at a concentration of 5000 cells/ml, full colonization was not inhibited in juvenile animals by the mannose analog, but was delayed up to 10 hr. These data suggest that inhibition by mannose analogs is only effective at concentrations of bacteria similar to those the animal experiences in its natural environment (Lee *et al.*, 1994).

We also found that the continued presence of the mannose analog is required to block infection by the bacteria. After the animals were exposed to bacteria in the presence of the mannose analog for 4 hr, the animals were placed in seawater without the sugar. These juveniles became infected, although the infection was delayed a few hours in comparison with the infection kinetics of the control groups. Also, when added after colonization, mannose analogs were not capable of eliminating the infection or changing the colonization level, even when added within a few hours of exposure to *V. fischeri* cells. Finally, animals that had been exposed to the mannose analog for 48 hr, rinsed and placed in seawater without analog, but containing 700 *V. fischeri* cells/ml, were colonized within 20 hr following exposure to symbionts. These data provide evidence that long periods of exposure to the sugar did not permanently alter the animal's ability to become colonized by its bacterial partner.

3. DISCUSSION

These data provide evidence for the presence of mannose-recognizing molecules on the surface of *V. fischeri* cells and mannose glycans on the apical surfaces of the host epithelia that interact with the bacteria during colonization. Colonization experiments with mannose analogs showed that this specific sugar is capable of inhibiting infection of the light organ, presumably by binding to the adhesins on *V. fischeri*, thereby blocking those sites from interaction with host cells. The final proof of the involvement of adhesin-glycan interactions in this symbiosis awaits the production of adhesin-defective mutants of *V. fischeri*.

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