Arg-Gly-Asp(RGD) peptides inhibit Streptococcus mitis to adhere to fibronectin

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Abstract: Fibronectin (Fn) is a multifunctional adhesive protein found on cell surfaces as well as in plasma. It is also believed to play an important role in bacterial adherence to host tissues. Molecular analyses of Fn have shown that the amino acid triplet arginine-glycine-aspartic acid (RGD) sequence functions as a binding site. We examined the role of the RGD sequence on bacterial adherence to Fn. The pretreatment of Streptococcus mitis with synthetic RGD-containing peptide reduced the number of bound bacteria to the Fn coated plates by 76%. In contrast, a control peptide containing the RGE sequence showed no inhibition. These data indicate that synthetic RGD peptides may be useful for the inhibition of bacterial adherence to Fn on host cell surfaces.

Key words: RGD peptide; fibronectin; Streptococcus mitis; inhibition of bacterial adherence.

Materials and Methods

S. mitis ATCC9811 was incubated in BHI broth at 37°C for 48h under an anaerobic condition (80 % N₂, 10 % H₂ and 10 % CO₂). After incubation, bacterial cells were centrifuged to collect, washed three times with phosphate-buffered saline (PBS, pH 7.4), and then suspended in PBS to an optical density of 0.2 at 540 nm. The following synthetic RGD-containing peptides were used: Arg-Gly-Asp (RGD), Arg-Gly-Asp-Ser (RGDS), Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP) (Telios Pharmaceuticals, San Diego, CA). The synthetic peptide Gly-Arg-Gly-Glu-Ser-Pro (GRGESP) (Telios Pharmaceuticals, San Diego, CA) was used as a control. Peptides were diluted in PBS. Bacterial cells were preincubated with peptides (0.1 mg/ml) at 37°C for 30 min (9). After preincubation, bacterial suspensions were transferred to Fn-coated plastic plates (Becton Dickinson Labware, Mississauga, Ontario) and further incubated at 37°C for 30 min. After incubation, the plates were washed 3 times with PBS and fixed in 1 % glutaraldehyde for 1h. Attachment of bacteria on the plates was enumerated at X5000 with a scanning electron microscope (JSM T100, Nihondenshi, Tokyo Japan). In each experiment, five isolated fields were examined. Experiments were repeated four times. Data were evaluated by the use of Student’s t test to determine statistically significant differences between each test and the control.

Results

Preincubation of S. mitis with the synthetic RGD, RGDS, and GRGDSP peptides resulted in a significantly lower number of bound bacterial cells onto the Fn-coated plates (-73.7 %, -73.7 %, and -76 %, respectively). In contrast, preincubation with the GRGESP peptide had no significant effect on adherence of S. mitis to the culture plates (-6.1 %) (Table 1).

Discussion

S. mitis, one of the earliest colonizers of human oral surfaces, has been isolated from subgingival plaque. Due to its multiple coaggregating nature, it lets several Gram-negatives and Gram-positives colonize on subgingival sites (10). It now appears that one of the primary functional consequences of the interaction of Fn with bacteria is in the initial adherence of the bacteria to host cells and tissues. Simpson et al. showed that Fn acted as a
receptor of oral epithelial cells for the adherence of Streptococci (11). In addition, Fn has been found in saliva as well as on the surfaces of oral epithelial cells (12,13). In this study, the treatment of S. mitis with synthetic RGD peptides inhibited bacterial adherence to Fn whereas the treatment with the control peptide, which contained an amino acid substitution in the RGD sequence (GRGESP), failed to decrease adherence of S. mitis. Therefore, we speculate that the receptor for S. mitis on Fn is an integrin-like molecule and specific to the RGD sequence of Fn. Leininger et al. (14) showed that the sequence of RGD was involved in the adherence of Bordetella pertussis to mammalian cells. The current findings suggest that synthetic peptides containing the RGD sequence may be useful as specific inhibitors against adhesion of S. mitis to oral cell surfaces.

Table: Inhibitory effects of pretreatment with various synthetic peptides on adherence of S. mitis to the Fn coated plates.

<table>
<thead>
<tr>
<th>Peptides</th>
<th>No. of Bacteria / Field</th>
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<tbody>
<tr>
<td>Control</td>
<td>201.3±35.23</td>
</tr>
<tr>
<td>Arg-Gly-Asp (RGD)</td>
<td>52.9±14.6</td>
</tr>
<tr>
<td>Arg-Gly-Asp-Ser (RGDS)</td>
<td>52.8±12.6</td>
</tr>
<tr>
<td>Gly-Arg-Gly-Asp-Ser-Pro</td>
<td>48.4±18.8</td>
</tr>
<tr>
<td>Gly-Arg-Gly-Glu-Ser-Pro</td>
<td>189.0±23.0</td>
</tr>
</tbody>
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* P < 0.001 Student's t test

References