Short Communication

Role of an Arginine Residue Present in Histatin 8 Which Inhibits Coaggregation between *Porphyromonas gingivalis* and *Streptococcus mitis*

Porphyromonas gingivalis と *Streptococcus mitis* との共凝集を阻害するヒスタチン 8 に
存在するアルギニン残基の役割

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Introduction

A family of histidine-rich polypeptides (HRPs) has been found to exist in human saliva and salivary glands¹⁻³⁾. The HRPs inhibit the growth of *Candida albicans*⁴⁾ and *Streptococcus mutans*⁵⁾ *in vitro*. Oppenheim *et al.*⁶⁾ named HRPs histatin, and Troxler *et al.*⁷⁾ have shown that in human parotid secretion there are at least twelve histatins which contain the common sequence of Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg. We have successfully purified histatin 8, Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg-Gly-Tyr⁸⁾.

Porphyromonas gingivalis that colonizes the oral cavity has often been shown to possess adhesive properties that enable it to bind to salivary pili-
cles, epithelial cells and Gram-positive bacterial

species⁹⁻¹¹⁾. We have reported a turbidimetric assay method to determine the coaggregation between *P. gingivalis* and *Streptococcus mitis*¹²⁾.

Our previous studies have shown that histatin 8 inhibits the coaggregation between *P. gingivalis* and *S. mitis*¹³⁾. However, it remains unclear whether the presence of the arginine residue in histatin 8 is actually responsible for the inhibition of coaggregation. We examined the inhibitory effects on coaggregation of shorter fragments of histatin 8 with and without an arginine residue.

Materials and Methods

P. gingivalis 381 was a gift from the Research Laboratory of Oral Biology, Sunstar Inc., Osaka, Japan. *S. mitis* ATCC 9811 was purchased from American Type Culture Collection (Rockville, MD). The bacterial cells were grown as described previously¹³⁾. They were centrifuged at 10,000×g for 20 min at 4°C, washed three times with 10 mM phosphate-buffered saline (PBS), pH 6.0, and resuspended in PBS. The suspension of *P. gingivalis* was adjusted to an absorbance of 1.0 at 550 nm (A₅₅₀), and *S. mitis* to 0.35. Five peptides, Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg-Gly-Tyr (peptide I: histatin 8), Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg (peptide II), Lys-His-His-Ser-His-Arg-Gly-Tyr (peptide III), Lys-His-His-Ser-His-Arg (peptide IV) and Lys-His-His-Ser-His (peptide V) were synthesized by a solid-phase technique using a peptide synthesizer 430 A (Applied Biosystems Inc., Foster City, CA). The reaction mixture for the coaggregation assay contained *P. gingivalis* 381 and *S. mitis* ATCC 9811 (each 0.5 ml containing 5 × 10⁸ cells), and 10 mM PBS, pH 6.0 or inhibitors in a final volume of 2 ml. The progress of coaggregation was monitored by measuring the decrease in A₅₅₀ with time using a UV-visible recording spectrophotometer and a temperature-controlled cuvette compartment with a magnetic stirrer (UV-265 FW, Shimadzu Co., Kyoto, Japan) at 37°C. The percentage of inhibition of coaggregation was calculated from the change of A₅₅₀ 5 min after the start of the reaction.

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Fig. 1 Inhibitory effect of the five synthetic peptides on coaggregation between *P. gingivalis* 381 and *S. mitis* ATCC 9811.

I : Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg-Gly-Tyr

II : Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg

III : Lys-His-His-Ser-His-Arg-Gly-Tyr

IV : Lys-His-His-Ser-His-Arg

V : Lys-His-His-Ser-His

Results

Fig. 1 shows the activity of the five peptides to inhibit the coaggregation between *P. gingivalis* 381 and *S. mitis* ATCC 9811. Coaggregation was strongly inhibited by peptides I and II, moderately by peptides III and IV, and far less by peptide V.

Discussion

We previously reported that basic peptides and protein, especially histatins 5 and 8 inhibit the coaggregation between *P. gingivalis* and *S. mitis*¹³⁾. In the present studies, we used shorter fragments of histatin 8. The peptide V fragment (Lys-His-His-Ser-His) which has no arginine residue was far less active than the other peptides, I to IV, which have an arginine residue. These findings suggest that the arginine residue is necessary for the inhibition of coaggregation.

P. gingivalis is well known to have highly pro-

teolytic activity and this bacterium generates a trypsin-like protease¹⁴⁻¹⁶⁾. Nishikata and Yoshimura¹⁷⁾ have purified a hemagglutinin (HA) with a molecular mass of 44 kDa from the membrane fraction of *P. gingivalis* 381. The HA possesses protease activity to hydrolyze proteins and arginine-containing synthetic substrates. Li *et al.* have reported that a trypsin-like enzyme of *P. gingivalis* is associated with its adherence to *Actinomyces viscosus*¹⁸⁾. Thus, *P. gingivalis* proteases also may contribute to the coaggregation between *P. gingivalis* and *S. mitis*. We have demonstrated that histatin 8 binds to *P. gingivalis* but not to *S. mitis*, and moreover, that the histatin 8 receptors on *P. gingivalis* have a molecular mass of about 44 kDa and 41 kDa protein^{13,19)}. More recently, Hiratsuka *et al.* reported that the 40 kDa outer membrane protein (OMP) is an important aggregation factor of *P. gingivalis*²⁰⁾. These findings indicate that the arginine residue in histatin 8 specifically interacts with the 44 kDa and/or 41 kDa OMP on the *P. gingivalis* surface.

Conclusion

The arginine residue in histatin 8 may have an important role in the inhibition of coaggregation between *P. gingivalis* and *S. mitis*.

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