Short Communication

Binding of Synthetic Histatin 8 to Oral Bacteria

合成ヒスタチン 8 の口腔細菌への結合

Yukitaka MURAKAMI*, Atsuo AMANO*,
Satoshi SHIZUKUISHI* and
Akira TSUNEMITSU*

村上 幸孝* 天野 敦雄*

雫石 聡* 常光 旭*

Introduction

Many novel families of histidine-rich polypeptides (HRPs) are present in human saliva and salivary glands¹⁻³⁾. The HRP have been shown to inhibit the growth of *Candida albicans*⁴⁾ and *Streptococcus mutans*⁵⁾ *in vitro*. In 1988, Oppenheim *et al.* named HRP histatin⁶⁾. Troxler *et al.* have demonstrated that there are at least twelve histatins in human parotid secretion⁷⁾. We also have successfully purified histatin 8, Lys-Phe-His-Glu-Lys-His-Ser-His-Arg-Gly-Tyr⁸⁾, but little attentions have been paid on the binding of histatin 8 to oral bacteria. In this communication, we describe that histatin 8 binds to *Porphyromonas gingivalis* specifically.

Materials and Methods

Bacterial strains and growth condition. *Porphyromonas gingivalis* 381, ATCC 33277, *Prevotella loescheii* ATCC 15930, *Prevotella melaninogenica* ATCC 25845, *Prevotella intermedia* ATCC 25261 and *Prevotella denticola* ATCC 33185 were supplied from the Research Laboratory of Oral Biology, Sunstar Inc., Osaka, Japan. *Capnocytophaga* AL 2-6-2 and *Eikenella corrodens* 375 were obtained

by the courtesy of Dr. H. Okada, Department of Periodontology and Endodontology, Osaka University Faculty of Dentistry. *Streptococcus cricetus* E 49, *Streptococcus rattus* FA-1, *Streptococcus mutans* NCTC 10449, MT703R, OMZ175, *Streptococcus sobrinus* B 13, 6715, *Streptococcus downei* MFe28, *Streptococcus mitis* ATCC 9811 and *Streptococcus sanguis* ATCC 10557 were generously provided by Dr. S. Hamada, Department of Microbiology and Oral Microbiology, Osaka University Faculty of Dentistry. The bacterial cells were grown as described previously^{9,10)}. They were centrifuged at 10,000×g for 20 min at 4 °C, washed three times with 10 mM Tris-HCl, pH 7.0, containing 0.15 M NaCl (TBS) and then used for binding assay. Cell numbers were determined using Bürker-Türk chamber, and the cell density was adjusted appropriately.

Synthesis of histatin 8. Histatin 8, Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg-Gly-Tyr, was synthesized by a solid-phase technique using a peptide synthesizer 430 A (Applied Biosystems Inc., Foster, CA) with a Boc-amino acid anhydride method. The synthetic sample was a gift from Dr. S. Aimoto, Institute for Protein Research, Osaka University.

Iodination of histatin 8. Histatin 8 was iodinated by previous our method¹¹⁾. The specific activity of the labeled histatin 8 was 2.6 mCi/μmol.

Binding of histatin 8. Binding assay was performed in 1.5 ml polypropylene microcentrifuge tubes coated with silicone to reduce the nonspecific binding of histatin 8. Bacterial cells (1×10^9 cells/tube) and ¹²⁵I-histatin 8 (5×10^4 cpm/tube) were incubated at 4 °C and 37 °C in 100 μl of TBS. After 120 min, cells were washed twice with TBS, and once with 10 mM Tris-HCl buffer, pH 7.0 containing 0.5 M NaCl. The radioactivity associated with pelleted bacteria was quantitated in a gamma counter (LKB WALLAC, Sweden). Specific binding was determined by subtracting the nonspecific binding in the presence of excess, unlabeled histatin 8 (1×10^{-7} mol/tube). The binding of histatin 8 to human red-blood cell was also determined by

* Department of Preventive Dentistry, Osaka University Faculty of Dentistry (Director: Prof. Akira TSUNEMITSU)

* 大阪大学歯学部予防歯科学教室 (主任: 常光旭教授)

1992年 3月30日受付

Table 1 Binding of ^{125}I -histatin 8 to oral bacteria

Strains	Specific binding (cpm)*	
	4° C	37° C
<i>Porphyromonas gingivalis</i> 381	1928	2892
<i>Porphyromonas gingivalis</i> ATCC 33277	1499	906
<i>Prevotella loescheii</i> ATCC 15930	656	1006
<i>Prevotella melaninogenica</i> ATCC 25845	2400	640
<i>Prevotella intermedia</i> ATCC 25261	1561	1036
<i>Prevotella denticola</i> ATCC 33185	1740	1674
<i>Capnocytophaga</i> AL2-6-2	1000	1188
<i>Eikenella corrodens</i> 375	325	237
<i>Streptococcus cricetus</i> E49 (a)**	54	171
<i>Streptococcus rattus</i> FA-1 (b)	257	80
<i>Streptococcus mutans</i> NCTC 10449 (c)	50	82
<i>Streptococcus sobrinus</i> B13 (b)	5	45
<i>Streptococcus mutans</i> MT703R (e)	675	248
<i>Streptococcus mutans</i> OMZ175 (f)	43	206
<i>Streptococcus sobrinus</i> 6715 (g)	57	282
<i>Streptococcus downei</i> MFe28 (h)	61	91
<i>Streptococcus mitis</i> ATCC 9811	305	306
<i>Streptococcus sanguis</i> ATCC 10557	92	6
Human red-blood cell	107	939

* Each value represents the average of duplicate determinations.

** Serotype

the same procedures as described in the binding assay to bacteria, using 1×10^9 cells/tube.

Results

The specific binding activity of ^{125}I -histatin 8 to oral bacteria tested is shown in Table 1. Variable differences in the binding between 4° C and 37° C were seen in the respective strains. *Porphyromonas*, *Prevotella*, and *Capnocytophaga* showed comparatively higher binding activity. However, little bindings were found in *Streptococcus* in spite of indicating small differences among the serotypes. The specific binding to human red-blood cell was insignificant in comparison with that of *P. gingivalis* 381.

Discussion

To reduce the nonspecific binding of ^{125}I -histatin 8, we used siliconized tubes. In addition, bacterial cells were washed with 10 mM Tris-HCl buffer con-

taining a high concentration (0.5 M) of NaCl. These methods have brought about better results. In this work, it has been shown that ^{125}I -histatin 8 clearly binds to anaerobic bacteria, especially to *P. gingivalis* 381 at both 4° C and 37° C. These findings suggest that histatin 8 interacts with *P. gingivalis* 381 cells through specific binding sites on their surface, and will allow the development of histatin 8 radioreceptor assay.

Pollock *et al.*⁽⁴⁾ have found that HRPs from human parotid saliva have a fungistatic and fungicidal activity on *Candida albicans* 18804, 28517 and 28815. Simultaneously, MacKay *et al.*⁽⁵⁾ have demonstrated the growth-inhibitory and bactericidal effects of human parotid salivary HRPs on *Streptococcus* GS-5, BHT and SB. In our laboratory, we have no strains of *C. albicans* and *S. mutans* described above, thus the specific binding assay of labeled histatin 8 to these bacteria will be investigated in the further studies.

Conclusion

Significant specific binding of ^{125}I -histatin 8 to *P. gingivalis* was found, but not to *S. mutans*, *S. mitis*, *S. sanguis* tested and human red-blood cells.

References

- 1) Oppenheim, F. G.: Salivary histidine-rich proteins, In J. O. Tenovuo (ed.), Human Saliva: Clinical Chemistry and Microbiology volume I, CRC Press, Inc., Florida, 1989, p. 151-160.
- 2) Sabatini, L. M., Warner, T. F., Saitoh, E. and Azen, E. A.: Tissue distribution of RNAs for cystatins, histatins, statherin, and proline-rich salivary proteins in humans and macaques, *J. Dent. Res.*, **68**; 1138-1145, 1989.
- 3) vanderSpek, J. C., Wyandt, T. F., Skare, J. C., Milunsky, A., Oppenheim, F. G. and Troxler, R. F.: Localization of the genes for histatins to human chromosome 4q13 and tissue distribution of the mRNAs, *Am. J. Hum. Genet.*, **45**; 381-387, 1989.
- 4) Pollock, J. J., Denepitiya, L., Mackay, B. J. and Iacono, V. J.: Fungistatic and fungicidal activity of human parotid salivary histidine-rich polypeptides on *Candida albicans*, *Infect. Immun.*, **44**; 702-707, 1984.
- 5) MacKay, B. J., Denepitiya, L., Iacono, V. J., Krost, S. B. and Pollock, J. J.: Growth-inhibitory and bactericidal effects of human parotid salivary histidine-rich polypeptides on *Streptococcus mutans*, *Infect. Immun.*, **44**; 695-701, 1984.
- 6) Oppenheim, F. G., Xu, T., McMillian, F. M., Levitz, S. M., Diamond, R. D., Offner, G. D. and Troxler, R. F.: Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on *Candida albicans*, *J. Biol. Chem.*, **263**; 7472-7477, 1988.
- 7) Troxler, R. F., Offner, G. D., Xu, T., vanderSpek, J. C. and Oppenheim, F. G.: Structural relationship between human salivary histatins, *J. Dent. Res.*, **69**; 2-6, 1990.
- 8) Murakami, Y., Amano, A., Takagaki, M., Shizukuishi, S., Tsunemitsu, A. and Aimoto, S.: Purification and characterization from human parotid secretion of a peptide which inhibits hemagglutination of *Bacteroides gingivalis* 381, *FEMS Microbiol. Lett.*, **72**; 275-280, 1990.
- 9) Shizukuishi, S.: Purification and some properties of α -L-fucosidase isolated from *Streptococcus sanguis* ATCC 10557: Role of the enzyme in degradation of salivary glycoprotein, *J. Osaka Univ. Dent.*, **23**; 1-16, 1978 (in Japanese).
- 10) Takeshita, T.: Purification and some properties of neuraminidase from the culture medium of *Bacteroides loeschei* ATCC 15930, *J. Osaka Univ. Dent.*, **34**; 322-342, 1989 (in Japanese).
- 11) Murakami, Y., Shizukuishi, S., Tsunemitsu, A., Nakashima, K., Kato, Y. and Aimoto, S.: Binding of a histidine-rich peptide to *Porphyromonas gingivalis*, *FEMS Microbiol. Lett.*, **82**; 253-256, 1991.

Key words: Histatin 8, Oral bacteria, Binding

索引用語: ヒスタチン 8, 口腔細菌, 結合性