ARCHIVES OF ORAL BIOLOGY 56 (2011) 869–876

available at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

Antimicrobial and DNA-binding activities of the peptide fragments of human lactoferrin and histatin 5 against *Streptococcus mutans*

Lijun Huo [a,b], *Kai Zhang* [b], *Junqi Ling* [a,b,*], *Zhixiang Peng* [b], *Xiangya Huang* [b], *Hongyan Liu* [b], *Lisha Gu* [b]

[a] *Institute of Stomatological Research, Sun Yat-sen University, 74 Zhong Shang Er Road, Guangzhou 510080, PR China*
[b] *Department of Operative Dentistry, Preventive Dentistry and Endodontics, Guanghua School of Stomatology, Sun Yat-sen University, 56 Ling Yuan Xi Road, Guangzhou 510055, PR China*

ARTICLE INFO

Article history:
Accepted 10 February 2011

Keywords:
Antimicrobial peptides
hLF1–11
P-113
Streptococcus mutans
DNA binding

ABSTRACT

Objective: To investigate the killing effect of two salivary antimicrobial peptides, hLF1–11 and P-113, and identify the antibacterial mechanism of the peptides.
Methods: The antimicrobial activities of hLF1–11 and P-113 against oral Streptococci strains were determined using the broth microdilution method. The effects of hLF1–11 and P-113 on the bacterial plasma membrane were visualized by scanning electron microscopy. Cell membrane permeability was monitored using the intracellular dye calcein. The subcellular localization of hLF1–11 and P-113 in bacteria was measured by fluorescence light microscopy. An electrophoretic mobility shift assay (EMSA) was performed to evaluate the DNA binding capabilities of hLF1–11, P-113 and MUC7 12-mer.
Results: Both hLF1–11 and P-113 exerted potent bactericidal activities against all selected oral Streptococci. *Streptococcus mutans* UA 159 was the most susceptible of the oral bacterial species tested to the antimicrobial effects of the three peptides. The cell membranes of bacteria treated with hLF1–11 or P-113 were still intact after 30 min. hLF1–11 and P-113 could penetrate the bacterial cell membranes and accumulate in the cytoplasm in *S. mutans*. Both hLF1–11 and P-113 showed DNA binding affinity.
Conclusions: Together, our results demonstrate that hLF1–11 and P-113 display antibacterial activity against dental cavity-inducing *S. mutans* through an intracellular mechanism that could involve DNA binding. Thus, these peptides might be attractive and valuable candidates for development into effective antimicrobial therapies to combat dental caries.

* *Corresponding author at*: Institute of Stomatological Research, Sun Yat-sen University, 74 Zhong Shang Er Road, Guangzhou 510080, PR China. Tel.: +86 20 8386 2621; fax: +86 20 8382 2807.
E-mail addresses: huolj@mail2.sysu.edu.cn (L. Huo), lingjq@mail.sysu.edu.cn (J. Ling).

Abbreviations: hLF1–11, human lactoferrin 1–11; P-113, a 12-amino-acid fragment of Hst5 (residues 4–16) containing a C-terminal amide group; *S. mutans*, *Streptococcus mutans*; AMPs, antimicrobial peptides; LF, lactoferrin; Hst, histatins; hLF, human lactoferrin; Hst5, histatin 5; MRSA, methicillin-resistant *Staphylococcus aureus*; MUC7 12-mer, a 12-amino-acid fragment of human salivary low molecular-mass mucin; FITC, fluorescein isothiocyanate; calcein-AM, calcein acetoxymethyl ester; *S. gordonii*, *Streptococcus gordonii*; *S. sanguis*, *Streptococcus sanguis*; BHI, brain heart infusion broth; MICs, minimal inhibitory concentrations; CFU, colony-forming unit; SEM, scanning electron microscopy; EMSA, electrophoretic mobility shift assay; *C. albicans*, *Candida albicans*; Arg, arginine.

0003–9969/$ – see front matter
doi:10.1016/j.archoralbio.2011.02.004

1. Introduction

Dental caries affect a large proportion of the world's population. *Streptococcus mutans* is considered the major etiologic agent involved in human dental caries. In past decades, efforts have been made to develop antimicrobial agents targeting *S. mutans* as a means of preventing dental caries. Chlorhexidine is a potent antimicrobial chemical agent; however, its unpleasant taste and dental discolouration hinder its clinical utility. Thus, there is a compelling need to develop alternative antimicrobial agents from natural sources with fewer side-effects.

Short, positively charged antimicrobial peptides (AMPs), which have been found in plants and animals,[1] are part of the innate immune system and help defend against invading microorganisms. Because they quickly kill a broad range of microorganisms, including species resistant to other antimicrobials, they have potential for development as new antibiotic agents.[2] In this regard, potent antimicrobials have been produced based on natural peptides and the active domains of larger antimicrobial proteins.[3]

Lactoferrin (LF)[4] and histatins (Hst)[5] are two families of human salivary proteins that contribute to innate immunity. Published data show that both human lactoferrin (hLF) and histatin 5 (Hst5) possess significant *in vitro* microbicidal activity against bacteria[6–8] and fungi.[9–11] To exert their antimicrobial effect, hLF and Hst5 bind to cell surface receptors, are internalized and interact with specific intracellular structures, such as DNA[12] and mitochondria,[13] without affecting bacterial membrane permeability.[14] Rather, the intracellular actions of AMPs disrupt the metabolic activity of infected cells, killing bacteria[8] and fungi.[13] Furthermore, shorter peptides derived from hLF and Hst5 also exert significant microbicidal activity against bacteria and fungi *in vitro*.[15,16] Two such examples are the antimicrobial peptide human lactoferrin 1–11 (hLF1–11) and P-113. The former is derived from the active domain of human lactoferrin (residues 1–11), and the latter is a 12-amino-acid fragment of Hst5 (residues 4–16) containing a C-terminal amide group. The peptide P-113 is a histatin derivative that is as potent as Hst5.[17] Both hLF1–11 and P-113 have a broad antimicrobial spectrum *in vitro* against both bacteria and fungi, including multi drug-resistant *Acinetobacter baumannii*, *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Candida albicans*.[6]

Both hLF1–11 and P-113 are the smallest fragments that retain antimicrobial activity comparable to their respective full-length proteins.[8,17] Although the bactericidal effect of oral salivary peptides has been proved, it is currently unknown whether the fragments hLF1–11 and P-113 kill oral Streptococci. Given the therapeutic potential of the AMPs, it is of interest to determine whether hLF1–11 and P-113 are capable of killing oral Streptococci more or less effectively than the full-length respective proteins. Thus, the objective of the present study was to investigate the susceptibility of selected oral bacteria to hLF1–11 and P-113. In addition, the effects of the two peptides on membrane permeability and on intracellular targets were studied in *S. mutans*.

2. Materials and methods

2.1. Peptides and chemicals

Lyophilized hLF1–11 (GRRRRSVQWCA), P-113 (AKRHHGYKRKFH), MUC7 12-mer (RKSYKCLHKRCR) (selected as a positive control peptide) were chemically synthesized with and without fluorescein isothiocyanate (FITC) conjugate, and then analysed for purity by Shanghai Invitrogen Biotech Ltd. (Shanghai, China). The purity of hLF1–11, P-113, MUC7 12-mer and their corresponding FITC-labelled forms were 96.78%, 96.13%, 95.66%, 95.33%, 96.48% and 97.12%, respectively. The purity of each peptide was taken into consideration in the preparation of stock solutions. The peptides were dissolved in sterile double-distilled (dd) water at 1.28 mg/ml. Aliquots (0.1 ml) were stored at −20 °C. Chlorhexidine acetate (Sigma Chemical Co., St. Louis, MO, USA) was dissolved to 1.0 mg/ml with sterile dd water. Plasmid DNA (pUC18) was purchased from Takara Biotech Co. Ltd. (Dalian, China). Calcein acetoxymethyl ester (calcein-AM) was obtained from Dojindo Molecular Technologies, Inc. (Dojindo, Japan). L-7012 LIVE/DEAD BacLight™ Bacterial Viability Kit was purchased from Molecular Probes (USA).

2.2. Bacterial strains and growth media

S. mutans UA 159 and *S. mutans* GS-5 were obtained from Research Institute of Stomatology, Sun Yat-sen University. *Streptococcus gordonii* ATCC 10558 and *Streptococcus sanguis* ATCC 49295 were obtained from China General Microbiological Culture Collection Center. Bacteria were cultured at 37 °C anaerobically overnight in brain heart infusion broth (BHI, Becton–Dickinson and Co.).

2.3. Bacterial susceptibility assay

The antimicrobial activities of hLF1–11, P-113 and MUC7 12-mer against oral Streptococci strains were investigated by serial microbroth dilution. Minimal inhibitory concentrations (MICs) of the peptides were determined using the broth microdilution method.[18] Bacteria were cultured anaerobically overnight in BHI broth at 37 °C, diluted 1:20 in fresh BHI and cultured anaerobically for 2.5 h to mid-logarithmic growth phase. Briefly, two-fold serial dilutions of each peptide were prepared in BHI medium at a volume of 200 μL per well in 96-well round-bottom microtitre plates (Costar, Cambridge, MA, USA). The final concentrations of hLF1–11, P-113 and MUC7 12-mer ranged from 0.73 to 91.12 μM, 0.64 to 81.12 μM and 0.63 to 81.84 μM, respectively. The final concentrations of chlorhexidine, used as a positive control, ranged from 0.12 to 15.99 μM. The wells of the microtitre plate were inoculated with 20 μL per well of bacterial cell suspension, at a final concentration of 1×10^5 CFU/ml for all bacterial species. After anaerobic incubation at 37 °C for 24 h, The MIC endpoint was defined as the lowest concentration of the test agents that completely inhibited growth compared with drug-free control. The MIC values are expressed as the median of at least three independent experiments.