

Inhibition of Protease Activity of Periodontopathogens by Purified Lactoferrin and Lactoferrin Supplement

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Abstract

Periodontitis is caused by periodontopathic bacteria such as *Porphyromonas gingivalis* and *Tannerella forsythia*. *P. gingivalis* has several biologic activities such as protease secretion. Inhibition of protease activities of periodontopathogens is one of the most effective strategies to prevent the progression of periodontitis. Lactoferrin, an iron-binding glycoprotein, is considered to be a useful tool for this purpose, and lactoferrin supplements may be effective for health promotion.

In this study, the effect of purified lactoferrin on *P. gingivalis* and *T. forsythia* protease was examined. The effect of lactoferrin supplement on protease activity was also tested. The purified lactoferrin inhibited the protease activity of both periodontopathogens. Lactoferrin supplement also exhibited the inhibitory effect. From these results, lactoferrin and lactoferrin supplement are considered to contribute to the prevention of periodontitis by inhibiting the protease activity of periodontopathogens.

Keywords: *P. gingivalis*; *T. forsythia*; Protease; Lactoferrin; Supplement

Introduction

Periodontitis is caused by periodontopathic bacteria such as *Porphyromonas gingivalis* [1] and *Tannerella forsythia* [2], which are components of so-called red-complex bacteria [3]. *P. gingivalis* has several biologic activities, and one of the most important properties is protease activity named gingipain [4]. We have previously isolated [5] and confirmed its virulence in a mouse mixed infection model with

P. gingivalis and *T. forsythia* [6]. Gingipain was also found to be related with the growth promotion of *P. gingivalis* by *T. forsythia* [7].

Inhibition of pathogenic activities of periodontopathogens is one of the most effective strategies to prevent the progression of periodontitis. Lactoferrin, an 80-kDa iron-binding glycoprotein of the transferrin family, is present in the milk, other exocrine secretions such as tears, and synovial fluid, and the secondary granules of neutrophils and blood [8]. In the oral cavity, it is secreted to saliva [9] and gingival crevicular fluid [10]. It is believed to play an important role in innate immunity, exhibiting antibacterial, antifungal, antiviral, antitumor, parasiticidal, immunomodulatory, and anti-inflammatory activities [11].

Materials and Methods

Bacterial strains and culture conditions

P. gingivalis was grown as previously reported [5]. *P. gingivalis* ATCC 33277 was maintained on CDC anaerobic blood agar (Becton Dickinson, Cockeysville, MD, USA) in an anaerobic atmosphere (80% N₂, 10% H₂, 10% CO₂).

Preparation of sonicated extracts of *P. gingivalis* cells

P. gingivalis cells of late logarithmic stage in tryptic soy broth were harvested by centrifugation and washed with phosphate-buffered saline (PBS). Fifty micrograms of these bacteria were suspended in 0.5 mL of PBS, and the cells were disrupted by sonication on ice [17]. Intact cells

Inhibition of *P. gingivalis* protease activity by lactoferrin

Lactoferrin was found to inhibit the protease activity of *P. gingivalis* in a dose-dependent manner (Figure 1). The inhibitory effect was observed both after 30 minutes and 60 minutes incubation. The lactoferrin supplement also inhibited the *P. gingivalis* protease activity, but the inhibition rate was lower than that of purified lactoferrin

Discussion

The importance of protease of periodontopathogens is well known [4]. It is closely associated with the destruction of periodontal connective tissues, disruption of host defense mechanisms, and development and maintenance of inflammation in periodontal pockets. We had previously reported the effect of gingipains on host defense system [5], mixed infection [6], and growth promotion by *T. forsythia* [7]. Inhibition of gingipain activity is considered to be a useful strategy for prevention of periodontitis, and new protease inhibitor was developed [19]. Periodontitis is widely spread among adults, and more easy method to suppress the protease activity is required. Lactoferrin, a multifunctional protein, is known to be safe to even children, and the supplement is widely used for health promotion.

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