Bacterial Adsorption to Fetuin and Mucin Pellicle

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Abstract

The ability of *Actinomyces viscosus* (*A. viscosus*) WVU 627 to attach to hydroxyapatite (HA) beads treated with either bovine fetuin or bovine mucin was studied. All preparations used were blocked with bovine serum albumin prior to incubation with [³H] thymidine-labeled *A. viscosus* cells. The quantity of fetuin or mucin adsorbed on the HA beads was determined by use of [³H] fetuin or [³H] mucin. Approximately 6 μ g protein of [³H] fetuin and 20 μ g of protein of [³H] mucin adsorbed to 5 mg of HA beads at saturation, respectively.

The presence of fetuin or mucin on HA beads promoted attachment of the organism. However, HA beads treated with human whole saliva as a positive control promoted *A. viscosus* attachment more effectively than HA beads treated with these glycoproteins. Attachment of two additional strains (B 236 and B 25) of *A. viscosus* to HA beads was also promoted by these glycoproteins. The number of *A. viscosus* cells which attached to fetuin-treated HA beads was dose-dependent, except for strain B 25. For all three *A. viscosus* strains tested, attachment to the experimental pellicle with mucin was dose-dependent.

These findings suggest that the use of these newly developed bacterial adhesion assay systems may be effective for elucidating bacterial adhesion mechanisms.

Introduction

Dental caries and periodontal diseases are caused by bacterial accumulations, called dental plaque, which develop on the teeth. For plaque bacteria, attachment to a tooth surface is the initial event in dental plaque formation^[1]. The mechanisms which mediate bacterial attachment are thought to include both specific and non-specific forces. Specific binding results from stereochemical interactions involving proteinaceous ligands, called "adhesins" on the bacterial surfaces and complementary molecules, called "receptors" on the host tissue^[2-4]. Many adhesins are associated with fimbriae or pilli, which bind to saccharide receptors^[2,5-7]. However, adhesins which bind to non-glycosylated proteins, such as the acidic salivary proline-rich proteins (PRPs) and statherin, have also been described recently^[8,9].

Actinomyces viscosus is a prominent plaque microorganism which preferentially colonizes the teeth and may require teeth for oral colonization, since it is not usually detected in the oral cavities of predentate infants^[10]. In adults, high proportions of this organism have been associated with gingivitis^[11,12] and root surface caries^[13,14]. Typical strains of *A. viscosus* possess two antigenically and functionally distinct types of fimbriae. *A. viscosus* is thought to attach to salivary pellicles on teeth via type 1 fimbriae^[15,16]. Type 2 fimbriae bind to galactosyl-containing receptors on mammalian cells or the surfaces of certain bacteria^[17,18]. Reactions involving type 2 fimbriae are inhibited by galactosides such as lactose, whereas type 1 fimbriae which mediate attachment to saliva-treated hydroxyapatite are not affected by such saccharides^[19,20].

Salivary acidic PRP and statherin adsorbed onto apatitic surfaces have recently been found to promote attachment of *A. viscosus*^[9]. The PRPs possess some segments which exhibit structural similarities to collagens^[9,21]. This structural relationship is of interest because collagen is a major component of the matrices

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of cementum and dentin, and collagen fibers become exposed when these tissues are subjected to acids produced by oral bacteria^[22,23]. The purpose of the present investigation was to devise a new bacterial adhesion assay system for use in elucidating bacterial adhesion mechanisms.

Materials and Methods

Bacterial strains and culture conditions

A. viscosus (WVU 627, B 236 and B 25) and *Actinomyces naeslundii* (ATCC 12104) were obtained from the culture collection of our laboratory. Stock cultures were stored in 50% glycerol at -20°C until used. All *A. viscosus* strains were preincubated in trypticase soy broth (Becton Dickinson Microbiology System, MD, USA) in anaerobic jars for 24 h at 37°C in an atmosphere of 95% N₂ and 5% CO₂. The fresh bacterial cells were then inoculated into trypticase soy broth supplemented by 92.5 kBq of [³H] thymidine (ICN Biochemicals, CA, USA) per ml. Bacteria used in the adhesion assay were grown to early stationary phase at 37°C under anaerobic conditions (BBL GasPak Anaerobic System, Becton Dickinson Microbiology Systems, MD, USA). Bacterial cells were harvested by centrifugation, washed twice and suspended in buffered KCl (0.05 M KCl containing 1 mM KH₂PO₄, 1 mM CaCl₂, and 0.1 mM MgCl₂ at pH 6.2) supplemented with 5 mg per ml of bovine albumin (Sigma Chemical Co., MO, USA). The suspensions were adjusted to contain 1x10⁸ (OD₅₅₀=0.10) bacteria per ml based on a standard curve relating optical density to the number of bacterial cells, as determined by microscopic counting.

Adhesion assays

Bacterial binding to glycoproteins (fetuin and mucin) was studied by use of glycoprotein-treated HA beads. Bacterial attachment was determined from experimental pellicles formed from whole saliva and soluble human type 1 placental collagen (Sigma) as a positive control, and from bovine fetuin (Boehringer Mannheim, Germany) and bovine mucin (Sigma) on spheroidal HA beads (BDH Chemicals, Gallard Schlessinger Chemical, NY, USA). Before assay, the beads were equilibrated overnight in buffered KCl at room temperature. Experimental pellicles were prepared by treating 5 mg of equilibrated HA beads with 125 µl of saliva, collagen, fetuin or mucin in microtitration plates. The mixtures were incubated for one hour at room temperature with continuous rotation (6 rpm). The beads were then washed twice with buffered KCl, and treated for 30 min with 5 mg per ml of bovine serum albumin in buffered KCl (BSA-KCl) to block any uncoated bead surface^[24]. The liquor was then removed, and the beads were incubated with ³H-labeled bacterial cells, using an adequate number of bacterial cells in 125 µl of BSA-KCl. After one hour of continuous rotation at room temperature, the beads were washed twice with buffered KCl, and transferred to scintillation vials. The number of cells that attached was determined by direct scintillation counting.

Preparation of saliva

Samples of whole unstimulated saliva were collected from a single adult donor in ice-chilled containers. The samples were heated successively to 56°C for 30 min to inactivate degradative enzymes, clarified by centrifugation at 2,000 xg for 20min, and filtered by a membrane (0.45 µm and 0.22 µm) (Nihon Millipore, Japan). The samples were then dialyzed for 3 days at 4°C in distilled water containing 0.004% NaN₃. The water was changed three times. The dialyzed samples were lyophilized and stored at -80°C until used.

Adsorption of [³H] fetuin and [³H] mucin to HA beads

To determine the quantity of glycoproteins which adsorbed to the HA beads, 5mg samples of HA beads were incubated with 125 µl of various concentrations of fetuin or mucin, which had been radiolabeled with [³H] formaldehyde as described by Jentoft and Dearborn^[25]. The [³H] glycoproteins were diluted with each of these unlabeled glycoproteins so as to contain 1,500 dpm per µg protein. The mixtures were incubated at room temperature for 1.5 h, and the beads then washed twice with buffered KCl and transferred to scintillation vials for counting.

Protein measurement

The protein content for each type of glycoprotein (fetuin and mucin) was estimated by the method of Lowry et al^[26]. Bovine serum albumin (Sigma) was used as a standard.

All assays were performed in duplicate, and most experiments were performed at least twice.

Results

Quantity of glycoproteins (fetuin and mucin) adsorbed to the HA beads

The quantities of glycoproteins which adsorbed to the HA beads were determined using [³H] fetuin and [³H]mucin. Approximately 6 μ g protein of fetuin and 20 μ g protein of mucin adsorbed to 5 mg of the HA beads at saturation (Figs. 1,2).

Attachment of A. viscosus cells to fetuin-treated HA beads

Treating HA beads with fetuin slightly promoted the attachment of *A. viscosus* WVU 627 and B 236 cells, but did not promote the attachment of strain B 25 cells. The attachment was dose-dependent. The cell count was higher for bacteria which bound to HA beads treated with saliva than for those treated with fetuin (Fig. 3).

Attachment of A. viscosus cells to mucin-treated HA beads

Treatment of HA beads with mucin also slightly promoted the attachment of cells of these three strains. The number of cells of these strains which bound to the experimental pellicle treated with mucin was almost the same as in the collagen-treated controls (Fig. 4).

Comparison of adsorption of A. viscosus and A. naeslundii cells to pellicles prepared from fetuin or mucin

The treatment of HA beads with either fetuin or mucin promoted the attachment of *A. naeslundii* ATCC 12104 cells rather more than that of *A. viscosus* WVU 627 cells (Figs. 5,6).

Discussion

Researchers studying the etiology of dental caries and periodontal diseases have recognized that these diseases are infections caused by bacterial plaque accumulations on the teeth. Efforts to identify the mechanisms involved in the formation of such dental plaques have revealed that bacterial attachment to tooth surfaces is a remarkably specific process, and that attachment is often the first step required for the colonization of teeth. The majority of oral bacteria display marked tissue tropisms. Thus, organisms such as *Streptococcus mutans*, *Streptococcus sanguis*, *A. viscosus*, and *Porphyromonas gingivalis* mainly colonize the teeth, whereas *Streptococcus salivarius* preferentially colonizes the tongue dorsum. *Streptococcus mitis* is found in high proportions on both buccal and tooth surfaces. It has become clear that bacteria possess a highly developed recognition system which can identify and interact with specific macromolecules present on tooth surfaces^[27].

A. viscosus is a major member of the microorganisms of human supra- and subgingival dental plaque. The organism preferentially colonizes the teeth, and it is thought that teeth are indispensable for oral colonization by the organism because it is not usually detected in the mouths of preterm infants^[10,28]. Typical strains of *A. viscosus* possess two distinct types of fimbriae (type 1 and type 2 fimbriae) which are thought to mediate its attachment to teeth^[17]. Type 2 fimbriae bind to galactosyl-containing receptors. Reactions involving type 2 fimbriae are inhibited by galactosides such as lactose. On the other hand, interactions involving type 1 fimbriae are not inhibited by the galactoside. *A. naeslundii* ATCC 12104 possesses only type 2 fimbriae^[15,29,30].

One of the most remarkable findings in the present study was that commercially purchased glycoproteins—bovine fetuin and bovine mucin—adsorbed to HA bead surfaces interacted with *A. viscosus* and *A. naeslundii* cells. These organisms either possess type 2 fimbriae, and were able to bind effectively to these glycoprotein pellicles. These pure glycoproteins have saccharide chains. Experimental pellicles which have these glycoproteins will next be used after degrading the saccharide chains with a degradable enzyme such as sialidase. This may reveal the lectin-like interactions existing between the bacteria and sugars.

These newly developed bacterial adhesion assay systems can be used widely as oral surface models for clarifying the specific mechanisms of bacterial attachment.

Conclusion

Several mechanisms are involved in the adsorption of oral bacteria to the surfaces of oral tissues. In this study, experimental pellicles treated with commercially purchased glycoproteins (fetuin and mucin) were developed for use as an oral bacterial adhesion assay system. Such systems can be widely utilized to identify the specific receptors responsible for bacterial adhesion.

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Fig. 1 Adsorption of ³H-fetuin to HA beads. Samples of HA (5 mg) were incubated with various amounts of bovine fetuin (1.0 μg/μl)(specific activity, 1,500 dpm/μg) for one hour.

Fig. 2 Adsorption of ³H-mucin to HA beads. Samples of HA (5 mg) were incubated with various amounts of bovine mucin (1.5 μg/μl)(specific activity, 1,500 dpm/μg) for one hour.

Fig. 3 Adsorption of *A. viscosus* (WVU 627, B 236 and B 25) to HA beads treated with bovine fetuin
The incubation mixtures contained various cell numbers of ³H-labeled bacteria (25-125 μ l: OD=0.40). 30 μ l (OD=0.40) of cell suspension was used for positive control pellicles (saliva and collagen).

Fig. 4 Adsorption of *A. viscosus* (WVU 627, B 236 and B 25) to HA beads treated with bovine mucin
The incubation mixtures contained various cell numbers of ³H-labeled bacteria (25-125 μ l: OD=0.40). 30 μ l (OD=0.40) of cell suspension was used for positive control pellicles (saliva and collagen).

Fig. 5 Comparison of adsorption of *A. viscosus* WVU 627 and *A. naeslundii* ATCC 12104 to HA beads treated with bovine fetuin

Fig. 6 Comparison of adsorption of *A. viscosus* WVU 627 and *A. naeslundii* ATCC 12104 to HA beads treated with bovine mucin