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Salivary gel-forming mucin MUC5B – a nutrient for dental plaque bacteria

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Introduction: Model systems with oral bacteria from dental plaque have demonstrated that the utilization of complex glycoproteins as a food source cannot be undertaken by single species but requires concerted degradation by a multi-species consortium, with each member contributing one or a few hydrolytic enzymes. Unlike previous studies, the aim of the present investigation was to explore the ability of fresh dental plaque to degrade salivary mucin, MUC5B, isolated by methods designed to retain intact the natural polymeric structure and physiological conformation, in an attempt to mimic the naturally occurring interaction between the oral microbiota and salivary mucins.
Methods: Human salivary MUC5B was isolated from whole saliva by density-gradient centrifugation and incubated with freshly isolated supragingival dental plaque with samples subjected to fluorescent staining for viability and metabolic activity. In addition, the degradation of MUC5B oligosaccharide side chains was studied using a lectin assay, recognizing three different carbohydrate epitopes commonly found on mucin oligosaccharide side chains.
Results: The addition of purified salivary MUC5B elicited a strong metabolic response from the biofilm cells, whereas individual strains of *Streptococcus oralis* and *Streptococcus gordonii* isolated from the same plaque were not able to utilize the MUC5B. The degradation of terminal saccharide moieties on the MUC5B was demonstrated by a marked decrease in both sialic acid and fucose reactivity.
Conclusion: These results have shown that dental plaque is capable of utilizing human salivary MUC5B as a nutrient source, a process possibly requiring the synergistic degradation of the molecule by a consortium of oral bacteria in the plaque community.

Key words: CTC; dental plaque; metabolic activity; MUC5B; mucindegradation

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Complex salivary glycoproteins are considered the major source of nutrients for the oral supragingival microbiota during the long periods of starvation between dietary intakes of carbohydrates by the host. As a consequence the indigenous microbial community has adapted to these conditions. A challenge for the resident oral microflora is to be able to degrade and utilize the complex carbohydrate structures provided by the salivary glycoproteins, a process requiring a wide range of glycosidases and proteolytic enzymes. Studies have identified sialidases in many species, including *Streptococcus oralis*, *Streptococcus intermedius*, *Streptococcus mitis*, and *Actinomyces naeslundii* (4, 6). *S. oralis* also expresses *N*-acetyl-β-D-glucosaminidase and β-D-galactosidase, in addition to α-L-fucosidase and mannosidase activity (7). Furthermore, mannosidase production has been identified within the viridans group streptococci (12) and fucosidase activity has been shown in *Lactobacillus rhamnosus* (6), while *Streptococcus sanguinis* and *S. oralis* also possess proteolytic activity (13).

In spite of such enzymatic activity, however, mono-species cultures have a poor capacity to utilize salivary glycoproteins as a nutrient source (11). Degradation of the complex oligosaccharide side chains of mucins is known to be highly dependent on interactions within consortia of different oral species displaying a complementary range of glycosidases and proteolytic activities. Such interspecies cooperation in the utilization of complex oligosaccharides

is also a factor in maintaining the diversity of the resident oral microflora (6, 32). This has been demonstrated in model systems, such as the chemostat, where pooled multispecies inocula were fed complex glycoproteins, such as hog gastric mucin and bovine submaxillary mucin, resulting in the development of multispecies communities capable of the synergistic degradation of the complex substrates (6, 8, 21, 32).

The dominant glycoproteins in saliva are the large oligomeric mucins and the major gel-forming mucin in saliva is MUC5B (22, 31, 35). MUC5B has a heterogeneous molecular mass as a result of its covalent polymeric structure (2000–40,000 kDa) and is heavily glycosylated. The glycosylation and the extreme size are crucial for gel formation because the disruption of this polymeric structure leads to the collapse of the mucous gel (5, 28). This gel-forming feature of mucin is considered one of the most important roles for the oligomeric mucins, coating epithelial surfaces throughout the body. In the oral cavity, this function is fundamental to both the adherence by oral bacteria to surfaces in the mouth and the provision of nutrients to the colonizing biofilms.

Earlier studies on the metabolic utilization of complex glycoproteins by oral bacteria used commercially available mucins, such as porcine gastric mucin and bovine submaxillary mucin (6, 8, 32), while human whole saliva has also been used as the sole nutrient to study cooperation in mixed-species biofilms (9, 23). In some of these studies, mucins have been processed by the reduction of disulfide bonds to facilitate soluble and stable solutions, thus using the mucin subunits as 'oligosaccharide carriers' (9, 23, 32). The abolition of the disulfide bonds, in addition to causing the collapse of the polymeric network, results in the exposure of protein epitopes and protein configurations, such as hydrophobic peptide domains and free thiol groups, that are not normally seen in the native molecule. This undoubtedly influences how the oral bacteria interact with the protein and its subsequent nutritional utilization.

The aim of the present investigation was to explore whether freshly isolated dental plaque was able to utilize human salivary MUC5B as a nutrient source. The objective was to purify human salivary MUC5B, keeping both the polymeric structure and the physiological conformation as intact as possible, in an attempt to mimic the naturally occurring interaction between the oral microbiota and salivary mucins.

Material and methods

Purification of human salivary MUC5B

MUC5B was isolated from human saliva and purified using a modified version of the method described previously (24). Whole saliva was collected on ice from eight individuals, pooled and then mixed with an equal volume of 0.2 M NaCl followed by incubation overnight with stirring at 4°C. The sample was then gently centrifuged at 4400 ***g*** for 30 min at 4°C and the supernatant was subjected to density-gradient centrifugation in CsCl/0.1 M NaCl (Beckman Optima LE-80K, rotor 50.2Ti, 36,000 r.p.m., 96 h, 15°C, start density 1.45 g/ml; Beckman, Fullerton, CA) and fractions were collected from the bottom of the tube. The fractions were analyzed for density (weighing) and for absorbance at 280 nm. MUC5B-containing fractions were identified using an antiserum against the peptide backbone of the protein (LUM5B-2), prepared as described by Wickström et al. (35), and the MUC5B-containing fractions were pooled and dialyzed against phosphate-buffered saline (PBS; 0.15 M NaCl, 5 mM NaH_2PO_4, pH 7) then stored at – 20°C until used. To determine the concentration of MUC5B, the MUC5B-containing fractions were pooled, dialyzed against water, lyophilized, and weighed. The pool of MUC5B was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), to examine whether low-molecular-weight proteins associated with the gel network contaminated the preparation.

Enzyme-linked immunosorbent assay (ELISA) and SDS–PAGE

ELISA was performed as previously described (35). In short, samples were coated on to multiwell assay plates (3912, Falcon, Franklin Lakes, NJ) overnight at room temperature. Plates were blocked for 1 h with PBS containing 0.05% (volume/volume) Tween-20 and 1% (weight/volume) bovine serum albumin (blocking solution) and incubated for 1 h with the LUM5B-2 antiserum diluted 1 : 1000. Detection was carried out using an alkaline phosphatase-conjugated swine antirabbit antiserum (Dako A/S, Copenhagen, Denmark), diluted 1 : 2000 in blocking solution with nitrophenyl phosphate (2 mg/ml in 1 M diethanolamine/HCl buffer, pH 9.8 containing 5 mM $MgCl_2$) as a substrate. Reactivity was expressed as absorbance at 405 nm after 1 h. SDS–PAGE was run using a Ready Gel® Precast gel (4–15% Tris–HCl) (Bio-Rad Laboratories, Hercules, CA) and the proteins were visualized using GelCode® Blue Stain Reagent (Pierce Biotechnology, Rockford, IL) following the manufacturer's instructions.

Bioassay for metabolic activity

For all experiments, 24-hour-old supragingival dental plaque was collected and pooled from the buccal and lingual surfaces of the premolar and molar regions of one 37-year-old healthy male before breakfast. The individual refrained from normal dental hygiene procedures the night before and on the morning of the collection, and the plaque sample was used for the experiments on the same day. The plaque was mixed by vortexing for 10 s and approximately 10 µl plaque was added to 1 ml MUC5B solution (0.3 mg/ml) in 1.5-ml tubes and incubated in air for 1 h in 37°C. The controls were plaque samples incubated with water rather than MUC5B. Following incubation, separate cell samples were subjected to staining for viability and metabolic activity using the BacLight™ LIVE/DEAD and RedoxSensor™ CTC Vitality Kits, respectively (Molecular Probes Inc., Eugene, OR). The BacLight LIVE/DEAD assay system contains two nucleic acid stains, SYTO® 9 and propidium iodide. SYTO 9 penetrates cells irrespective of the status of the cellular membranes, whereas propidium iodide only penetrates cells with damaged membranes, staining the cells red. For LIVE/DEAD viability assays, 30-µl aliquots of the dental plaque suspension were incubated for 10 min with 20 µl mixture of SYTO 9 and propidium iodide in the dark at room temperature followed by examination under a fluorescent microscope. CTC staining involved the redox fluorescence dye, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), targeting active products of cellular respiration, together with a DNA-staining dye (SYTO® 24). Although CTC is recognized as an indicator of bacterial respiration it is suggested that CTC is reduced at different sites depending on the respiratory chain used by the organism. For instance, in the facultative anaerobic *Escherichia coli* it is reduced by nicotinamide-adenine dinucleotide diphosphate [NAD(P)H] (17). For the CTC/SYTO 24 procedure, 30-µl aliquots of the plaque suspension were incubated with 20 µl dye (20 mM CTC and 1 µl/ml SYTO 24) for 30 min in the dark at room temperature before microscopic analysis.

Using the Model DS-2 Mv Nikon Digital Sight system (Nikon Corp., Tokyo, Japan), fluorescent microscopic images were captured with a digital camera connected to a personal computer with the software NIS-Elements BR 2.30 (Nikon Corp.). For each experiment, 10 different microscopic fields were documented with one representative image of each experiment shown in the results. To calculate the proportion of viable/non-viable cells and metabolically inactive/active cells, respectively, the per cent surface coverage of fluorescent green and red was determined using the software MATLAB, v.7.0 for Windows. As an overall control, the metabolic activities of mono-species cultures of *Streptococcus gordonii* and *S. oralis* grown on blood agar were determined using the procedure described above. In addition, *S. gordonii* and *S. oralis* were incubated in glucose-containing Todd–Hewitt broth (Difco, Nordic Biolabs, Stockholm, Sweden) and then incubated with the CTC/SYTO® 24 kit as a control for the ability to reduce CTC.

Glycosidase activity of dental plaque

The glycosidase activity of the supragingival dental plaque in suspension was assayed using fluorogenic (4MU-linked) substrates in a 96-well microtitre plate (Nunc Maxisorb, Roskilde, Denmark). The substrates used were β-D-glucoside, α-D-glucoside, α-L-arabinopyranoside,*N*-acetyl-β-D-glucosaminide, *N*-acetyl-*O*-D-neuraminide, α-L-fucoside, β-D-fucoside, β-D-galactoside, α-L-galactoside, and *N*-acetyl-β-D-galactosaminide. Dental plaque was collected in 50 mM *N*-Tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid (TES) buffer, pH 7.5 and 50 µl of the plaque suspension was mixed with 20 µl of each substrate (100 µg/ml in 50 mM TES buffer). The plates were incubated for 5 h at 37°C and absorbance was recorded in a fluorimeter fitted with a 96-well plate reader, at excitation and emission wavelengths of 355 and 460 nm, respectively.

Analyses of MUC5B oligosaccharide side chain degradation

Degradation of MUC5B oligosaccharide side chains was demonstrated by a lectin assay, using the *Sambucus nigra* lectin recognizing sialic acid (NANA-2-6), the *Ulex europaeus* agglutinin I lectin recognizing fucose (α-fuc) and the *Helix pomatia* lectin recognizing *N*-acetylgalactosamine (α-GalNAc). Approximately 10 µl plaque was added to 1 ml MUC5B solution (0.3 mg/ml) in an Eppendorf test tube and incubated for 4 h at room temperature. The bacteria were removed by gentle centrifugation (4400 ***g*** for 2 min) and the supernatant was coated on to 96-well assay plates (3912, Falcon) overnight at room temperature. Plates were blocked for 1 h with PBS containing 0.05% (volume/volume) Tween-20 and 1% (weight/volume) bovine serum albumin (blocking solution) and incubated (1 h) with biotinylated lectin (Vector Laboratories, Burlingame, CA) (2 µg/ml) diluted in blocking solution with 0.1 mM $CaCl_2$. Detection was carried out using alkaline phosphatase-conjugated streptavidin (Vector Laboratories, Burlingame, CA) with nitrophenyl phosphate (2 mg/ml in 1 M diethanolamine/HCl buffer, pH 9.8 containing 5 mM $MgCl_2$) as a substrate. Reactivity was expressed as absorbance at 405 nm after 1 h. Antibody reactivity (LUM5B-2) was used as an internal control to correct for MUC5B that had settled out as sediment as the result of bacterial adhesion.

Identification of microbial groups

After collection and mixing, the supragingival dental plaque samples were inoculated onto prereduced blood agar plates and incubated anaerobically for 7 days. The total number of colony-forming units was counted, paying special attention to the morphology, size, and number of different colony types. Cells from representative colonies of each morphological type were stained with Gram stain and inoculated on blood agar for later identification. Gram-positive, catalase-negative cocci in chains were considered to be streptococci and identified to species and subspecies level based on previously described characteristics (3, 14, 33). Gram-negative cocci were identified as *Veillonella* spp. based on size, obligatory anaerobic growth stimulation by lactate, but not glucose, and the production of acetate and propionate as the major acid end products from growth on lactate (10). Isolates growing on blood agar incubated in 5% carbon dioxide were considered facultative anaerobic microorganisms.

Results

Mucin purification

Human salivary MUC5B was isolated from whole saliva by density-gradient centrifugation. Using the peptide-specific antiserum LUM5B-2, the MUC5B-containing fractions were identified in the density gradient with the UV-absorbing low-molecular-weight material enriched at the top of the gradient (Fig. 1). MUC5B-containing fractions were pooled as shown in Fig. 1 and subjected to an SDS–PAGE to establish purity (insert, Fig. 1). As expected for pure MUC5B, staining was observed only in the well and no other staining was seen. The MUC5B concentration was estimated to 0.3 mg/ml following lyophilization and weighing. This pool of purified human salivary MUC5B was used for the following experiments.

Composition and glycosidase activity of dental plaque

In this study, freshly collected dental plaque was used to elucidate whether plaque biofilm cells could utilize human salivary MUC5B as a nutrient source. The microbial composition of this plaque, as determined following growth on blood agar and identification based on characteristics described in Material and methods, is

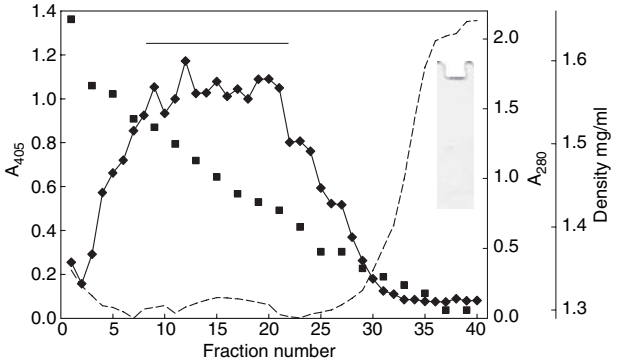

Fig. 1. Density-gradient centrifugation in CsCl/0.1 M NaCl of whole saliva 'extracted' with NaCl. Samples were emptied from the bottom of the tubes and fractions were analyzed for density (■), UV_{280} (––) and antibody reactivity with LUM5B-2 (♦). The fractions containing MUC5B were pooled, dialyzed against PBS and subjected to SDS–PAGE (insert).

Table 1. Microbial composition of natural dental plaque from one individual

Organism	Percentage of total cultivable microbiota[1]
Gram-positive cocci	53
Streptococcus oralis	36
Streptococcus cristatus	5
Streptococcus gordonii	2
Other facultative anaerobes	10
Gram-negative cocci	2
Veillonella sp.	2
Gram-positive rods	25
Actinomyces naeslundii	13
Other facultative anaerobes	12
Gram-negative rods	20
Capnocytophaga spp.	3
Leptotrichia spp.	2
Anaerobes	15

[1]Yield of known oral bacteria following growth on prereduced blood agar expressed as a percentage of the total counts.

summarized in Table 1. The major components comprising the total cultivable microbiota of the plaque samples were the gram-positive cocci (53%) and rods (25%) with *S. oralis* (36%) and *Actinomyces naeslundii* (13%) as the predominant organisms. *S. cristatus* and *S. gordonii* accounted for 5% and 2% of the total cultivable microbiota, respectively, while other facultative anaerobic gram-positive cocci comprised 10%. *Veillonella* spp. dominated the gram-negative cocci observed, but only accounted for 2% of the flora, while 20% of the total count was identified as gram-negative rods, including *Capnocytophaga* spp. and *Leptotrichia* spp.

A range of glycosidase substrates were used to investigate the glycosidic activity of the dental plaque suspensions collected and the results are summarized in Table 2. The predominant enzymatic activities were shown to be glucosidases and β-*N*-acetylglucosaminidase with additional strong reactivity of α-L-fucosidase, β-galactosidase, β-*N*-acetylgalactosaminidase, sialidase, and α-L-fucosidase. The latter two are of special interest because terminal fucosylated and sialylated structures are commonly found on MUC5B oligosaccharides. No activity was found for α-arabinosidase, β-fucosidase, α-galactosidase.

Table 2. Glycosidase activity in natural dental plaque

Enzyme	Activity
α-arabinosidase	−
α-L-fucosidase	+
β-D-fucosidase	−
α-galactosidase	−
β-galactosidase	+
α-glucosidase	+
β-glucosidase	+
β-*N*-acetylgalactosaminidase	+
β-*N*-acetylglucosaminidase	+
Sialidase (neuraminidase)	+

Increased metabolic activity in the presence of MUC5B

Freshly collected plaque suspensions were incubated with MUC5B for 1 h and then stained with BacLight LIVE/DEAD reagents measuring membrane integrity to assess viability. At the same time, separate samples of the same plaque suspension were assayed for metabolic activity with CTC/SYTO 24 dye. The results of the LIVE/DEAD staining procedure showed that there was essentially no difference between cells incubated without (Fig. 2A) and with (Fig. 2C) MUC5B. Computer-assisted numerical analysis of the images showed that 98% (95–99%) of the controls without MUC5B were viable, while 95% (90–99%) of those incubated with MUC5B were viable. Staining for metabolic activity with the CTC/SYTO 24 dye indicated that the control cells incubated in water (Fig. 2B) exhibited only 3% (1–7%) metabolically active red cells in all of the fields examined. By contrast, 70% (60–99%) of the plaque cells incubated with MUC5B (Fig 2D) were red, indicating active metabolism.

As a result of the described mode of action of the CTC stain, a control experiment using *S. oralis* and *S. gordonii* isolated from the collected plaque samples incubated in glucose-containing Todd–Hewitt broth was carried out, showing that these strains were able to reduce CTC (Fig 3, insert). Comparable experiments to the plaque study in Fig. 2 were carried out with *S. oralis* and *S. gordonii*. Typical results from *S. oralis* (Fig. 3) showed that the controls without MUC5B exhibited green, viable cells when stained with the BacLight viability stain (Fig. 3A) and metabolically inactive cells when stained with the CTC/SYTO 24 (Fig. 3B). The addition of purified MUC5B to each of the isolated strains led to little or no change in either membrane integrity or metabolic activity as typified by *S. oralis* in Fig. 3C,D.

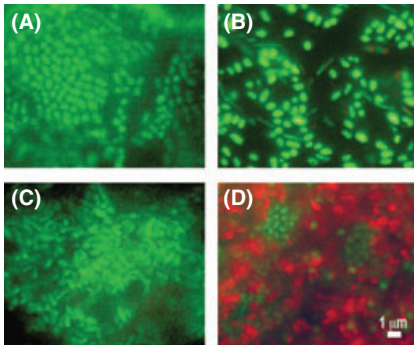

Fig. 2. Freshly collected dental plaque incubated for 1 h without MUC5B (A, B) and with MUC5B (C, D). Samples were stained with a BacLight viability kit (for membrane integrity) (left panel) and CTC/SYTO24 (for metabolic activity) (right panel). The experiment was repeated three times and for each experiment 10 different microscopic fields were documented. One representative image is shown.

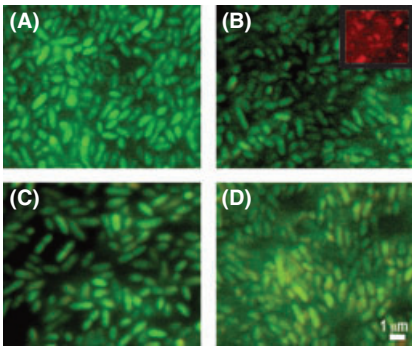

Fig. 3. Streptococcus oralis strain isolated from freshly collected dental plaque, stained with BacLight viability kit (for membrane integrity) (left panel) and CTC/SYTO24 (for metabolic activity) (right panel). *S. oralis* incubated for 1 h without MUC5B (A, B) and with MUC5B (C, D). The experiments were repeated three times and for each experiment 10 different microscopic fields were documented. One representative image is shown. Insert shows control indicating the ability of *S. oralis* to reduce CTC.

Degradation of MUC5B oligosaccharides by natural dental plaque

To determine degradation of MUC5B oligosaccharide side chains by dental plaque, lectins recognizing specific carbohydrate moieties were used. The lectins employed were the *S. nigra* lectin recognizing sialic acid (NANA-2-6) and the *U. europaeus* agglutinin I lectin recognizing fucose (α-fuc), both carbohydrates commonly found as terminal residues on the MUC5B oligosaccharide side chains. The *H. pomatia* lectin recognizing *N*-acetylgalactosamine (α-GalNAc), the core residue binding to the peptide backbone, was also used. As seen in Fig. 4 (black bars), MUC5B was shown to contain the structures tested. After incubation with natural dental plaque for 4 h a decrease of both sialic acid and fucose was observed, suggesting degradation of terminal sugar

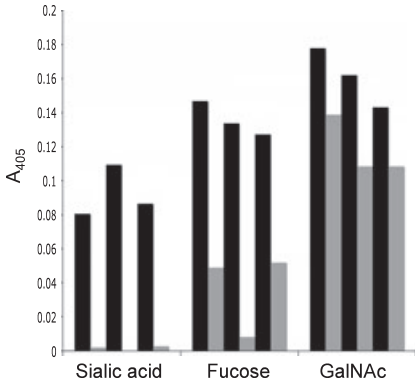

Fig. 4. Degradation of MUC5B oligosaccharide side chains. MUC5B was incubated without (black bars) and with (gray bars) freshly collected dental plaque for 4 h. Samples (triplicates) were analyzed for lectin reactivity [*Sambucus nigra* lectin recognizing sialic acid (NANA-2-6), *Ulex europaeus* agglutinin I lectin recognizing fucose (α-fuc) and *Helix pomatia* lectin recognizing *N*-acetylgalactosamine (α-GalNAc)]. The experiment was repeated three times.

residues by the oral biofilm cells (Fig. 4, gray bars). The reactivity with the GalNAc-lectin *H. pomatia* was decreased less after incubation.

Discussion

The results in this paper demonstrate that natural dental plaque is able to utilize, unlike isolated strains of *S. oralis* and *S. gordonii* from the same plaque, and degrade the complex oligosaccharide side chains of the human salivary MUC5B mucin. The environmental conditions for the dental plaque biofilm are characterized by factors like pH, redox potential, and essential nutrients that are required to uphold the distinct composition of the community (19). The composition of an individual oral biofilm is a result of a dynamic balance between inter-microbial and host–microbial interactions (1, 18). Saliva molecules in the acquired pellicle or 'conditioning film' on the tooth surface provide the initial attachment sites for colonizing bacteria during plaque formation. Salivary proteins and glycoproteins, such as mucins, can influence the establishment and selection of the oral microflora promoting adhesion of certain bacteria or facilitating clearance by aggregation and swallowing of other species (e.g. 19, 27).

In addition to plaque formation, endogenous salivary glycoprotein serves as a nutrient source for the plaque microflora during the long daily periods between the consumption of dietary fermentable carbohydrates (2, 16, 20). To degrade and utilize these glycoproteins as a nutrient source, a consortium of bacterial species is needed because no single bacterial species possesses the necessary range of hydrolytic enzymes. For example, when *S. mutans* was incubated with bovine serum albumin alone, little proteolytic activity was evident, however, when *S. oralis* was added, a significant increase in the degree of degradation was observed (11). Earlier studies have developed multispecies communities using pooled inocula in chemostats (21) to investigate the growth of the consortium from the energy derived from the synergistic degradation of complex glycoproteins, such as, hog gastric mucin and bovine submaxillary mucin (e.g. 6, 7, 32). In the current study, freshly collected natural dental plaque exhibited significantly increased metabolic activity within 1 h when introduced to human salivary mucins (MUC5B), whereas isolated strains of *S. oralis* and *S. gordonii* from the same plaque were unable to utilize the mucins as a nutrient source. The short incubation period also indicates that the complex plaque microbial community already possessed the range of hydrolytic enzymes required to degrade the mucins, while the isolated and cultured cells, grown overnight on blood agar, did not.

One of the aims of this study was to use MUC5B with both the polymeric structure and the physiological conformation as intact as possible in an attempt to mimic the physiological situation in the mouth. In our earlier characterization studies, MUC5B had been extracted using guanidinium hydrochloride (34, 35); however, Raynal and colleagues showed subsequently that MUC5B extracted with guanidinium hydrochloride reduces the capacity to form gel structures (24, 25). To preserve the structure of the salivary MUC5B, we isolated the mucins without guanidinium hydrochloride using a modified version of the method described (24). To our knowledge, this is the first attempt to study the interplay between human salivary mucins and freshly collected natural dental plaque, using oligomeric mucins purified in a non-denatured state. Salivary MUC5B is known to exist in subpopulations differing in both charge and 'solubility' and it has been postulated that differences in 'solubility' may influence the macromolecular 'architecture' of mucins (30, 34, 35). The organization of the salivary conditioning film, specifically the architecture of the mucins, may be more complex than was earlier believed and this suggests that future studies should consider mucins more than carriers of oligosaccharides.

When purified MUC5B was introduced to natural dental plaque in this study, degradation of terminal oligosaccharide structures was evident by the loss of fucose and sialic acid reactivity, as seen in Fig. 4. The *O*-glycans on MUC5B (MG1) have been described as very heterogeneous with a unique repertoire of oligosaccharides compared to the other secreted salivary mucin MG2 (MUC7) (15, 26, 29). Very large *O*-linked oligosaccharides, containing approximately 40 monosaccharides, have been found (29) and have been coupled with the large MUC5B molecule to form an extraordinarily complex and diverse glycoprotein. Studies of the carbohydrate composition have revealed mainly type 1 and 2 core structures (Galβ1-3GalNAcα1- and Galβ1-3GlcNAcβ1-6GalNAcα1, respectively) and have shown that fucosylated structures were more abundant than sialylated ones (molar ratio 5 : 1) (29). This is in agreement with our results that the reactivity with the fucose-specific lectin *U. europaeus* I was slightly higher than the *S. nigra* lectin, recognizing sialic acid. The reactivity of the core residue (GalNAc) seemed to decrease to a lesser extent suggesting that at the same time that degradation occurred, more of these structures were exposed after introducing biofilm cells to the mucins, because of the cleavage of more terminal saccharides. Alternatively, the incubation time chosen for this study was insufficient for the consortia to degrade the whole oligosaccharide. Nevertheless, from the results in this study, it is evident that the dental plaque is capable of degrading very complex glycoproteins, such as the salivary MUC5B.

Acknowledgments

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