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Collagen Mediates Adhesion of *Streptococcus mutans* to Human Dentin

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Some strains of *Streptococcus mutans* were found to recognize and bind collagen type I. Binding of ^{125}I-labeled collagen type I was specific in that collagen types I and II, but not unrelated proteins, were able to inhibit binding of the labeled ligand to bacteria. Collagen binding to *S. mutans* was partially reversible and involved a limited number of bacterial binding sites per cell. *S. mutans* UA 140 cells bound collagen type I with high affinity ($K_d = 8 \times 10^{-8}$ M). The number of binding sites per cell was 4×10^4. Collagen-binding strains of *S. mutans* were found to adhere to collagen-coated surfaces as well as to pulverized root tissue. *S. mutans* strains that did not bind the soluble ligand were unable to adhere to these substrata. Adherence to collagen-coated surfaces could be inhibited with collagen or clostridial collagenase-derived collagen peptides. Adherence of *S. mutans* to dentin was enhanced by collagen types I and II but inhibited by collagen peptides. *S. mutans* UA 140 bound significantly less ^{125}I-collagen type I following treatment with peptidoglycan-degrading enzymes. These enzymes released a collagen-binding protein (collagen receptor) with a relative molecular size of 16 kDa. The results of this study suggest that collagen mediates adhesion of *S. mutans* to dentin. This interaction may target collagen-binding strains of *S. mutans* to dentin in the oral cavity and may play a role in the pathogenesis of root surface caries.

Several species of bacteria have been implicated in the etiology of root surface caries. The mutans streptococci, actinomycetes, and lactobacilli are isolated more frequently and in larger numbers from root surface lesions than from noncarious exposed root surfaces (7, 10, 11, 17, 18, 37, 39; reviewed in reference 6). A recent study (31) demonstrates that suppression of streptococci alone in the root surface microbiota arrests the progression of root caries. Taken together, these data suggest that mutans streptococci, also implicated as etiologic agents of coronal caries, play a central role in the development of root surface caries. Root caries is a problem of increasing clinical importance, yet the molecular mechanisms critical to its development remain an enigma.

Bacterial adherence is a prerequisite for colonization and infection of a susceptible host. It is known that mutans streptococci adhere to tooth surfaces via insoluble glucan formation as well as by glucan-independent mechanisms involving adhesins that recognize salivary components present in the acquired enamel pellicle (reviewed in references 4 and 24). *Streptococcus mutans* may use these mechanisms to adhere to both enamel and dentin surfaces. Most studies examining *S. mutans* adherence have used hydroxyapatite and saliva-coated hydroxyapatite as a models for enamel and saliva-coated enamel, respectively. These models are appropriate for examining the interactions of *S. mutans* with enamel of the crowns of teeth but may be inappropriate for examining interactions with the dentin of exposed root surfaces. While dentin and enamel are both mineralized tissues, dentin is less mineralized and has a unique organic matrix composed mainly of collagens, with noncollagenous proteins and proteoglycans as minor components. Collagen type I is the predominant constituent, but other collagen types (III, V, and VI) have also been reported (review in reference 26). Enamel and dentin are distinctly different tissues and likely represent distinctly different substrata for bacterial adhesion. The possibility that the initiation and progression of root caries are mediated by specific interactions of cariogenic bacteria with components of exposed root surface dentin has not been previously explored. Recent studies suggest that collagen is recognized by oral streptococci and, when coupled to an apatitic surface, may serve as an adhesion substratum (27, 28). Streptococci that possess the ability to recognize and adhere to collagen may be specifically targeted to root surfaces and may have at their disposal an adhesion mechanism lacking in strains that do not recognize collagen. While interactions between collagen and streptococci may be modulated initially by salivary components, this modulation would likely be unimportant at the advancing front of a carious lesion in dentin. In such locations, the ability to attach to collagen may be an important virulence factor for streptococci. The aim of this study was to characterize the interactions between collagen type I and human isolates of *S. mutans* and to evaluate the potential importance of this interaction in adherence of *S. mutans* to human dentin.

MATERIALS AND METHODS

Bacteria. Strains of *S. mutans* were isolated from humans as part of a mother-infant transmission study. Characterization of these strains has been the subject of previous reports (8, 9). Bacterial strains were maintained at −70°C, and the number of passages was kept to a minimum. Bacteria were grown anaerobically in Todd-Hewitt broth (Difco) to early stationary phase in an atmosphere of 80% N_2, 10% H_2, and 10% CO_2 at 37°C. Cultures were centrifuged, resuspended, washed in phosphate-buffered saline (PBS; 0.14 M NaCl–50

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mM phosphate [pH 7.2] containing 0.01% sodium azide), and adjusted to a density of 10^{10} cells per ml, using a standard curve matching the A_{600} of the bacterial suspension to the number of bacterial cells counted microscopically. For adhesion experiments, bacteria were surface labeled with ^{125}I in a lactoperoxidase-catalyzed reaction as described previously (36). Specific radioactivity of labeled bacteria typically ranged from 5×10^2 to 2×10^3 cells per cpm, and suspensions remained stable for at least 2 weeks.

Chemicals. Purified human and bovine collagen type I (placental), extracted by limited pepsin digestion, was obtained from Southern Biotechnology Associates (Birmingham, Ala.), collagen type II (bovine nasal cartilage) was a gift from Edward Miller, University of Alabama at Birmingham, and partially purified porcine skin collagen (gelatin) was from Sigma. Human fibrinogen was obtained from Kabi (Stockholm, Sweden); human fibronectin was obtained from New York Blood Center (New York, N.Y.); α_1-acid glycoprotein and bovine serum albumin (BSA) were obtained from Sigma. Proteins used in binding assays were labeled with ^{125}I, using the chloramine T method as described elsewhere (23). Labeled proteins were analyzed by gel electrophoresis, and their migration patterns were compared with those of corresponding unlabeled proteins.

Digestion of collagen with *Clostridium histolyticum* collagenase was performed according to the procedure of Stark and Kühn (32). Digestion was performed for 18 h at a collagen-to-enzyme ratio of 10:1. The electrophoretic pattern of digested collagen (data not shown) showed an almost complete disappearance of α chains, which migrated as bands of apparent molecular size above 100 kDa on reducing gels, and the appearance of a wide spectrum of smaller components, below 50 kDa.

Digestion of bacteria. Bacteria (5×10^9 cells) in 10 mM Tris-HCl buffer (pH 7.2) were digested for 3 h at 37°C with 10 μg of mutanolysin per ml and 500 μg of lysozyme per ml. The suspension was then centrifuged, and the supernatant (lysate) was stored at −20°C.

Electrophoresis and blotting. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed as described by Blobel and Dobberstein (5). Unless otherwise stated, the samples were boiled in a buffer containing 4% SDS, 2 mM dithiothreitol, 30% sucrose, and 0.01% bromophenol blue in 82 mM Tris-HCl and then subjected to alkylation with iodoacetamide. The gels were stained with Coomassie brilliant blue R-250 and dried. Blotting was done as described by Towbin et al. (38). Proteins separated by electrophoresis were electrotransferred at 4°C for 2 h at 200 mA onto nitrocellulose (Schleicher & Schuell, Keene, N.H.). Unoccupied binding sites were blocked by incubating the membrane with 1% BSA in PBS for 30 min. The membrane was then incubated with 5×10^5 cpm of ^{125}I-labeled collagen type I in PBS containing 0.1% BSA and 0.1% Tween 80 for 2 h, washed with several changes of PBS containing 0.1% Tween 80, dried, and autoradiographed with X-ray film (Fuji, Japan).

Bacteria–^{125}I-collagen binding assay. The details of the standard assay have been described elsewhere (16). Briefly, bacterial cells (2.5×10^7) were incubated with ^{125}I-labeled collagen (approximately 5×10^4 cpm) in a total volume of 0.5 ml of PBS containing 0.1% BSA and 0.1% Tween 80. The tubes were rotated end over end for 2 h at 20°C unless otherwise stated. The reaction was stopped by the addition of 3 ml of ice-cold PBS containing 0.1% Tween 80, and the mixture was centrifuged (1,500 × *g*, 20 min). The supernatant was aspirated, and the radioactivity associated with bacteria was determined with a gamma counter.

Adhesion assays. (i) Collagen-coated substratum. Removable microtiter wells (Immulon 3; Dynatech Laboratories, Chantilly, Va.) were coated overnight with 50 μl of collagen type I (0.1 mg/ml) and then subjected to blocking with 200 μl of 0.1% BSA for 2 h. The wells were then overlaid with a 50-μl suspension of ^{125}I-labeled bacteria (5×10^6 cells per ml). Bacteria were allowed to attach to the coated wells for 2 h at 37°C. Wells were then extensively washed with PBS containing 0.1% Tween 80 and individually counted in the gamma counter.

(ii) Tooth root particles. Root particles were prepared as follows. Human teeth, mostly noncarious third molars, were thoroughly freed of adherent tissue by using a curette. The roots were then separated from the crowns of the teeth by using a diamond separating disk mounted on a dental lathe. Roots were cut into roughly uniform pieces and ground with a commercially available grinder. Particles were then sifted through a series of sieves. Fractions ranging in size from 90 to 125 μm were pooled and used as an adhesion substratum. In our standard assay, root particles were suspended in incubation buffer (PBS containing 0.1% Tween 80 and 0.1% BSA) at a concentration of 40 mg/ml. The suspension was vigorously mixed, and 50 μl (2 mg) of particles was added to 450 μl of incubation buffer containing ^{125}I-labeled bacteria (5×10^6 cells per ml) and incubated for 2 h at 37°C with end-over-end mixing, unless stated otherwise. The root particles were allowed to sediment by gravity, washed twice with 3 ml of ice-cold PBS containing 0.1% Tween 80 to remove unbound bacteria, and counted in the gamma counter. The surface area of the particles was calculated by assuming a spherical shape and a diameter of 100 μm. The possible contribution of porosity to the surface area of the particles was disregarded in these calculations. Using these assumptions, we calculated the surface area of 2 mg of root particles (the amount present in one assay tube) to be 73 mm^2. All calculations were performed on the basis of this assumption. Reported data are averages of at least two experiments, each performed in quadruplicate.

RESULTS

Collagen binding by *S. mutans*. In our initial experiments, 55 randomly selected strains of *S. mutans* were screened for the ability to bind ^{125}I-labeled collagen type I derived from a variety of sources, including human placenta, bovine placenta, and porcine skin (as gelatin). These strains were isolated from adult patients with a variety of ethnic backgrounds. Our data (Fig. 1) indicate that 11 of 55 strains tested, or 20%, bound ^{125}I-labeled collagen type I derived from human placenta. These 11 strains, but not the other 44 strains, also bound collagen type I derived from bovine placenta and porcine skin (gelatin). The amount of collagen bound by collagen-binding strains, henceforth referred to as positive strains, was comparable to the amount of collagen bound by collagen receptor-positive strains of *Staphylococcus aureus* tested in a previous study (35). Collagen type I derived from human placenta was used throughout the remainder of the study. The amount of collagen bound by positive strains did not appear to be affected by the growth medium used; i.e., positive strains bound the same amount of collagen regardless of whether they were grown in Todd-Hewitt broth, in brain heart infusion broth, or on blood agar (data not shown). Moreover, positive strains did not lose the ability to bind collagen after repeated subculturing, suggest-

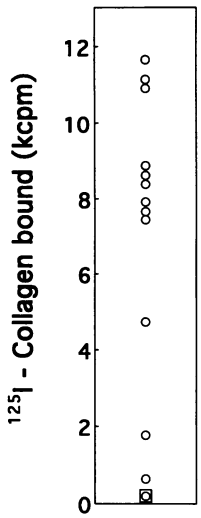

FIG. 1. Screening of randomly selected strains of *S. mutans* for the ability to bind ^{125}I-labeled placental collagen type I. Bacteria were tested for the ability to bind collagen in the standard assay with 4.1×10^4 cpm of labeled collagen. Each circle denotes one strain. The bottom circle in a square represents 43 strains which bound less than 200 cpm of added ^{125}I-labeled collagen type I.

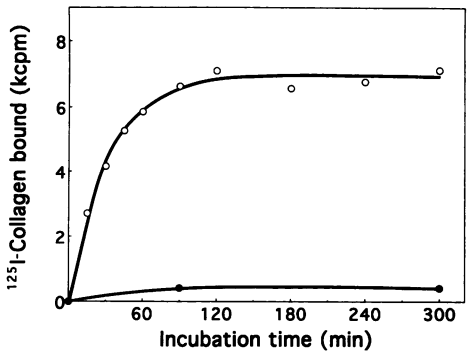

FIG. 2. Kinetics of binding of ^{125}I-collagen type I to *S. mutans* UA 140 (○) and UA 430 (●). The ability of bacteria to bind ^{125}I-labeled collagen as a function of time was determined in the standard assay.

ing that the collagen binding phenotype is probably not regulated by environmental conditions. Two strains of *S. mutans*, UA 140 (positive strain) and UA 430 (collagen nonbinder, negative strain), were selected for further characterization of collagen binding.

Binding of ^{125}I-labeled collagen to bacteria was saturable and essentially complete after 2 h of incubation (Fig. 2). This incubation time was used in all subsequent experiments. The amount of bound collagen remained stable after prolonged incubation, indicating that there was no degradation of the ligand or its receptor. The kinetics of binding as well as the maximal amount of bound ligand remained unchanged regardless of whether bacteria used in the assay were alive, heat treated (60°C, 20 min; data not shown), or killed with azide, as shown in Fig. 1. Addition of collagenase inhibitors had no effect on the amount of collagen bound by the bacteria. Binding occurred over a broad range of pH values and was essentially the same within a pH range of 6.0 to 8.0 (data not shown). For consistency, azide-killed, frozen bacterial suspensions were used throughout these experiments.

Binding of collagen type I to bacteria was specific, because it was inhibited, in a concentration-dependent manner, by an excess of unlabeled collagen type I (Table 1). Nearly complete inhibition of binding was achieved by addition of 10 μg of unlabeled collagen to assay mixtures (20 μg/ml, final concentration). Virtually the same degree of inhibition was achieved when collagen type II was used as a competing ligand. This may be explained by the high degree of similarity between these two collagen types. Binding of collagen to bacteria was unaffected by the presence of a variety of other proteins at concentrations 10^2-fold higher than that of collagen type I, further attesting to the specificity of collagen binding. With the exception of fibrinogen, which slightly inhibited collagen binding, there was also no effect when these proteins were present at concentrations 10^3-fold higher than that of collagen. Since none of the streptococcal strains tested, including *S. mutans* UA 140, were able to bind ^{125}I-labeled fibrinogen (33), it is unlikely that fibrinogen inhibited collagen binding by competing for binding sites on the surface of the bacteria. The effect of fibrinogen on collagen binding is probably due to an interaction between the two ligands. Binding of collagen by bacteria appears to be at least partially reversible, since more than half of bound ^{125}I-collagen type I was displaced by a 10^3-fold excess of unlabeled collagen type I (Table 1). Collagen types I and II were equally effective as displacing proteins, suggesting that a common element in these collagens may be recognized by the bacteria. Noncollagenous proteins were unable to displace bound collagen.

Incubation of bacteria in the presence of increasing concentrations of collagen resulted in increased binding of the ligand to bacteria (Fig. 3). At collagen concentrations ex-

TABLE 1. Specificity and reversibility of binding of ^{125}I-collagen to *S. mutans* UA 140[a]

Protein	Competition (% ± SD)		Displacement (% ± SD)	
	1 μg	10 μg	1 μg	10 μg
Collagen type I	23.6 ± 3.3	6.7 ± 2.1	61.3 ± 3.7	44.4 ± 3.3
Collagen type II	24.3 ± 4.2	5.4 ± 2.0	65.7 ± 4.6	42.7 ± 3.1
Fibrinogen	89.0 ± 7.4	77.3 ± 4.9	103.8 ± 8.6	92.4 ± 8.8
Fibronectin	96.6 ± 6.4	89.9 ± 6.1	110.2 ± 7.1	105.9 ± 4.8
Fetuin	92.9 ± 9.8	98.4 ± 7.2	102.3 ± 5.8	103.7 ± 8.7
α_1-Acid glycoprotein	99.3 ± 9.5	93.8 ± 5.2	105.5 ± 8.0	103.0 ± 7.8
Nonimmune immunoglobulin G	100.2 ± 5.8	97.9 ± 4.5	105.8 ± 8.9	98.9 ± 7.8

[a] Expressed as percentage of binding relative to controls (bacteria incubated in the absence of displacing or competing proteins). In competition experiments, competing proteins were added to the incubation mixtures simultaneously with ^{125}I-labeled collagen. In displacement experiments, bacteria were incubated with ^{125}I-collagen for 2 h as in the standard assay and then incubated for 2 h in the presence of the indicated amounts (per 0.5 ml) of displacing proteins.

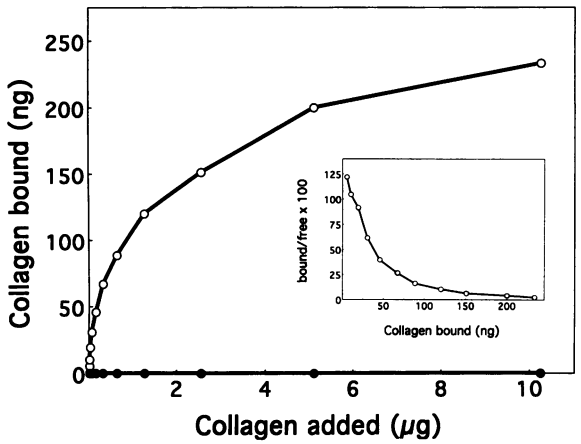

FIG. 3. Saturability of ^{125}I-labeled collagen binding to *S. mutans* UA 140 (○) and UA 430 (●). Insert, Scatchard plot analysis of data referring to strain UA 140. Bacteria (5×10^7 cells) were incubated with increasing amounts of ^{125}I-labeled collagen type I (specific activity, 7.6×10^5 cpm/μg). Background values were determined for each concentration of added collagen by omitting bacteria from incubation mixtures. Background radioactivity was subtracted from the total radioactivity determined at each collagen concentration tested.

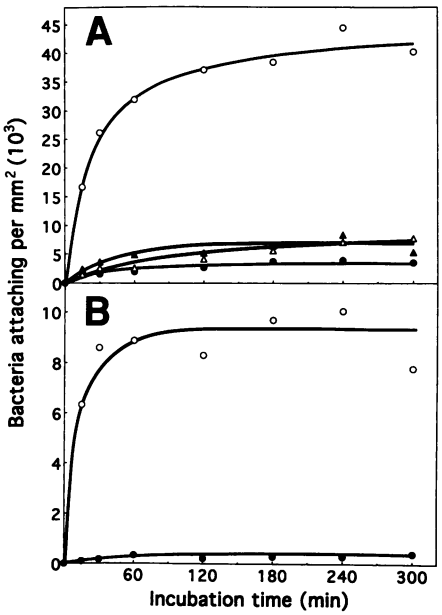

FIG. 4. Kinetics of adherence of two strains of *S. mutans* to collagen-coated microtiter wells (A) and human tooth root particles (B). Bacteria, surface labeled with ^{125}I, were incubated in wells or with root particles as described in Materials and Methods. The number of bacteria adhering as a function of time was determined. (A) Adherence to microtiter wells of *S. mutans* UA 140 (○, collagen; △, BSA) or UA 430 (●, collagen; ▲, BSA). (B) Adherence to tooth root particles of *S. mutans* UA 140 (○) and UA 430 (●).

ceeding 10 μg/0.5 ml, bacteria tended to clump and form aggregates, which consistently resulted in skewed saturation curves. Therefore, data obtained with use of higher concentrations of collagen were not included in Fig. 3. Clumping of bacteria in the presence of higher concentrations of collagen may indicate that each collagen molecule contains multiple binding sites for bacteria and that collagen can act as a bridge spanning two or more streptococcal cells. Other proteins, such as fibronectin and fibrinogen, have been shown to clump bacterial cells that bind these proteins (12, 20). The flattening of the saturation curve (Fig. 3) was taken as an indication of saturation. Saturability of binding indicates that bacteria possess a limited number of binding sites for collagen on their surfaces. Assuming that the maximal level of binding shown in Fig. 3 is saturating, we calculated that 5×10^7 cells bound 230 μg of collagen. Using 2.85×10^5 as the molecular weight of collagen type I (13) and assuming a single occupancy model, we calculate that each bacterial cell possessed approximately 10^4 collagen binding sites. Scatchard analysis (30) of the binding data (Fig. 3, insert) gave a downward convex line. In the past, we (15) have resolved this type of plot into two straight lines corresponding to two classes of binding sites. Using this approach, we calculated that *S. mutans* UA 140 expressed surface receptors binding collagen with high affinity ($K_d = 3.3 \times 10^{-10}$ M [2.8×10^3 binding sites per cell]) and lower affinity ($K_d = 7 \times 10^{-9}$ M [8.1×10^3 binding sites per cell]), for a total of 1.1×10^4 available binding sites per cell. However, the curvilinear appearance of the Scatchard plot may be due to collagen-collagen interactions (21, 25), as well as complex bacteria-collagen interactions (clumping of bacteria by collagen) which become apparent at high collagen concentrations. In this case, there may only be one class of binding sites for the collagen ligand on streptococcal cells. For a one-site model, the affinity of collagen type I for bacterial receptors may be taken as that concentration of collagen saturating one-half of the receptors ($K_d = 8 \times 10^{-9}$ M). It is difficult to exclude the possibility that bacteria possess two classes of collagen binding sites differing in their affinities, in view of the fact that 45% of collagen type I bound by bacteria could not be displaced by an excess of the ligand (Table 1). It may be possible to resolve this issue in the future if less complex forms of the ligand, i.e., collagen peptides, that bind bacteria with high affinity yet do not promote bacterial aggregation or self-association can be identified.

Role of collagen binding in bacterial adhesion. The ability of some strains of *S. mutans* to bind collagen type I with high affinity prompted us to evaluate the role of this interaction in bacterial adhesion. Ideally this role should be evaluated by using isogenic mutants. Since these were not available, we compared attachment of two strains of bacteria, a collagen-binding positive strain (UA 140) and a nonbinding negative strain (UA 430), to surfaces coated with either collagen type I or BSA. The results (Fig. 4A) indicate that collagen-binding cells attach to collagen-coated surfaces. Adhesion of the positive strain to albumin-coated surfaces was much lower and comparable to the adhesion of the negative strain to both collagen and albumin (considered to be nonspecific adherence). Adherence was time dependent and essentially complete within 2 h. Adherence was saturable, and at saturation, approximately 4×10^5 bacterial cells adhered per mm^2.

The ability of bacteria to adhere to dentin may depend on recognition of multiple dentin components. We examined the ability of strains UA 140 (positive) and UA 430 (negative) to attach to dentin particles. The data in Fig. 4B show that strain UA 140 attached to dentin particles whereas strain UA 430 did not. These data also indicate that adherence of bacteria to dentin is more rapid than that to collagen-coated surfaces and reaches equilibrium within 30 min. The same proteins which were tested as inhibitors of binding of ^{125}I-labeled collagen to bacterial cells (Table 1) were also tested as inhibitors in both adherence assays. Noncollagenous

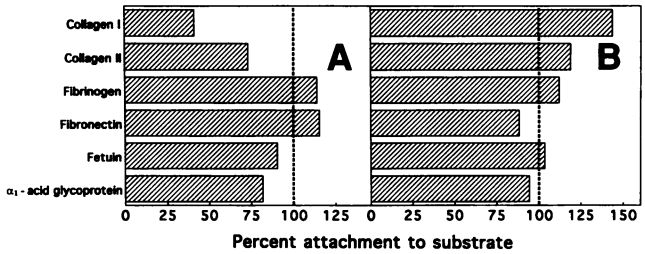

FIG. 5. Influence of various proteins on the adherence of *S. mutans* UA 140 to collagen-coated microtiter wells (A) and human tooth root particles (B). Bacteria (5×10^8 cells), surface labeled with ^{125}I, were preincubated with 10 μg of the proteins designated on the left for 1 h, after which they were incubated for 2 h either in collagen-coated wells or with root particles as described in Materials and Methods. Data are expressed as percentage of adherence relative to controls (bacteria incubated in the absence of proteins).

proteins did not significantly inhibit bacterial attachment to either collagen-coated surfaces (Fig. 5A) or root particles (Fig. 5B). Preincubation of bacteria with collagen type I substantially inhibited adherence of bacteria to collagen-coated microtiter wells, whereas collagen type II was less effective (Fig. 5A). Interestingly, both collagen type I and collagen type II enhanced adherence of bacteria to dentin (Fig. 5B). These results suggest that collagen may interact in a complex manner not only with bacteria but also with specific adhesion substrata, in this case, dentin. To test this hypothesis, we performed a series of experiments in which digested collagen, rather than the intact molecule, was used to pretreat bacteria prior to use in adhesion assays. The results (Fig. 6) clearly demonstrate that preincubation of bacteria with a mixture of collagen peptides inhibits bacterial attachment to collagen-coated surfaces as well as to dentin in a concentration-dependent manner. These results suggest that bacteria recognize a discrete region of the collagen molecule that is preserved in collagenase-digested collagen. In addition, these results suggest that the ability of streptococcal cells to adhere to dentin may be highly dependent on the ability of these bacteria to recognize dentin collagen.

Identification of a collagen adhesin. The high affinity of collagen for positive strains of *S. mutans* suggested that we might be able to identify a collagen receptor on nitrocellulose blots following separation of a cell extract by SDS-PAGE. To this end, bacterial cells were digested with a mixture of mutanolysin and lysozyme. Following this treatment, the amount of collagen bound by bacteria decreased to half of that of controls. The bacterial lysate, obtained after centrifugation to remove cells, contained a mixture of proteins which could be separated by SDS-PAGE (Fig. 7, lane a). When nitrocellulose blots were probed with ^{125}I-labeled collagen, a broad band, molecular size in the range of 16 kDa, was visualized by autoradiography (Fig. 7, lane b). This component may represent a collagen receptor of *S. mutans*, or at least its active portion. This band was not present in lysate of the negative strain (Fig. 7, lane c), nor it was detected in lysate of *S. mutans* UA 140 cells, which were trypsin treated prior to digestion and rendered incapable of binding ^{125}I-labeled collagen. The electrophoretically separated receptor bound lower amounts of ^{125}I-labeled collagen than did the original digest. Dot blots of unseparated bacterial lysate consistently bound more ^{125}I-collagen than did the 16-kDa component separated by electrophoresis. This finding was consistent regardless of whether the lysate was treated with SDS or reducing agents or whether samples were boiled prior to dot blotting. There are several possible explanations for these findings. The receptor may be conformationally altered and exhibit decreased affinity for collagen following electrophoresis and electroblotting, access of collagen to the blotted material may be limited, and/or the electrophoretic separation may result in removal of a cofactor necessary for stabilization of the receptor. Given that the receptor can be solubilized in an active form from bacterial cells by using only peptidoglycan-degrading enzymes (mutanolysin and lysozyme), and that trypsin digestion irreversibly destroys the ability of bacteria to interact with collagen (data not shown), it seems likely that the collagen receptor is a protein.

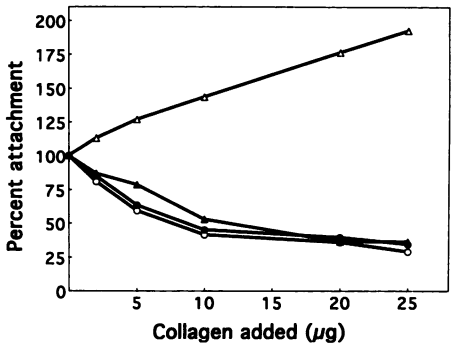

FIG. 6. Influence of collagen type I and collagenase-digested collagen type I on the adherence of *S. mutans* UA 140 to collagen-coated microtiter wells and human tooth root particles. Increasing concentrations of collagen or its collagenase digest were incubated with bacteria for 1 h, after which they were incubated for 2 h either on collagen-coated wells or with root particles. Symbols represent adherence to microtiter wells of bacteria incubated with collagen (○) or collagen digest (●) and adherence to root particles of bacteria incubated with collagen (△) or collagen digest (▲).

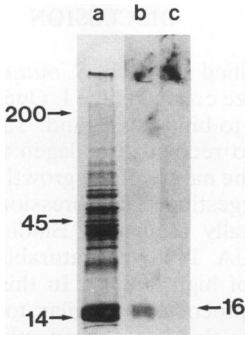

FIG. 7. Identification of a *S. mutans* UA 140 collagen-binding protein (collagen receptor). A lysozyme-mutanolysin digest of bacteria was separated by PAGE in SDS (5 to 10% linear acrylamide gradient). One portion of the gel was stained with Coomassie blue G (lane a), and the other was electrotransferred to nitrocellulose, blocked with 1% BSA, probed with ^{125}I-labeled collagen (5×10^5 cpm) for 2 h, washed, dried, and autoradiographed (lane b). Lane c, lysate of negative strain *S. mutans* UA 430, separated by electrophoresis, blotted, and probed with ^{125}I-labeled collagen. Migration distances and molecular sizes (in kilodaltons) of selected standard proteins are shown on the left; the arrow on the right indicates the migration distance and relative molecular size of the collagen-binding protein.

DISCUSSION

We have identified strains of *S. mutans* which possess the ability to recognize collagen type I. One-fifth of the 55 strains tested were able to bind this ligand. The ability of collagen-binding strains to recognize collagen type I was not influenced by either the nature of the growth medium or repeated subculturing, suggesting that expression of this phenotype is not environmentally regulated. Binding of collagen to *S. mutans* strain UA 140 was saturable, specific, partially reversible, and of high affinity. In this respect, binding of collagen to streptococci is similar to binding of the cell adhesion proteins (laminin, fibronectin, and fibrinogen) to other bacteria (K_d in the range of 10^{-8} to 10^{-10} M) (22). Our data suggest that studying the interaction of streptococci with collagen either as a soluble ligand or in collagenous substrata may be complicated by the tendency of collagen molecules to self-associate and form oligomers, as well as the tendency of soluble collagen type I to associate with other components in complex substrata such as dentin. In this latter property, collagen may be similar to fibronectin (21).

We have used two in vitro model systems in an attempt to evaluate the role of binding of collagen type I in adhesion of *S. mutans* to collagen-containing substrata. The results indicate that a collagen-binding strain of *S. mutans* was able to adhere to both collagen-containing substrata, while a nonbinding strain was not. Adhesion of a collagen binding strain of *S. mutans* to collagen-coated polystyrene wells was inhibited by soluble collagen type I and to a lesser extent collagen type II, whereas adhesion of this strain to human root dentin was enhanced by these ligands. We hypothesized that these apparently contradictory results are due to complex interactions of soluble collagens with dentin which do not occur when artificial, homogeneous substrata, such as collagen-coated polystyrene wells, are used as an adhesion model. Alternatively, collagen in dentin may be in a conformation that promotes collagen-collagen interactions, whereas collagen adsorbed to polystyrene wells is not. These results underscore the difficulties encountered when attempts are made to move from simple to more complex in vitro models for studying bacterial adherence to ligand-containing surfaces. Fortunately, it appears that the binding site for *S. mutans* on the collagen molecule is preserved in collagenase-derived collagen peptides, thus allowing us to demonstrate that collagen present in dentin is likely the sole mediator of the direct binding of *S. mutans* to root particles.

Although numerous interactions between bacteria and connective tissue components have been reported, in only a few instances (34) has a role for these interactions in bacterial adhesion or tissue tropism been convincingly demonstrated. Our data indicate that collagen-binding strains of *S. mutans* also recognize the ligand in dentin. A role for collagen binding in adherence of streptococci has been suggested in other recent studies (27, 28), in which strains of *Streptococcus cricetus* and *S. rattus* were shown to attach to collagen coupled to an apatitic surface. Adherence of *S. mutans* and *S. sobrinus* was also examined in this study; 2 of 15 strains (13%) were reported to adhere to collagen coupled to hydroxyapatite, which is in agreement with the frequency we have observed here. The influence of salivary components on the interaction of *S. mutans* with dentin remains to be determined. In fact, several salivary components, including salivary glycoprotein (1) and the proline-rich proteins, which share substantial sequence homology with collagen (19), have been shown to bind to certain strains of mutans streptococci. It has also been proposed that components of crevicular fluid, such as fibronectin, can mediate attachment of *S. mutans* to root surfaces, since *S. mutans* has been reported to attach to immobilized fibronectin (2, 3). Although none of the *S. mutans* strains tested in our laboratory bound soluble fibronectin, it is possible that fibronectin adsorbed to root surfaces can interfere with recognition of dentin collagen by *S. mutans*.

S. mutans UA 140 appears to bind collagen type I via a proteinaceous component. Streptococci treated with mutanolysin and lysozyme bound only half of the ^{125}I-labeled collagen bound by controls, whereas streptococci treated with trypsin were unable to bind ^{125}I-collagen. The mutanolysin-lysozyme lysate did not competitively inhibit binding of ^{125}I-collagen to intact streptococcal cells. In spite of this, a 16-kDa component was identified in an SDS-PAGE-separated lysate of strain UA 140, blotted and probed with ^{125}I-collagen. This collagen-binding component may be a collagen receptor or represent the active portion of the receptor. It was not found in mutanolysin-lysozyme lysates of nonbinding strains. Similar data were obtained when a receptor mediating binding of *S. aureus* to collagen type II was identified (35). The *S. aureus* collagen receptor was present, though not active as an inhibitor of collagen binding to bacteria, in lysostaphin lysates of *S. aureus*. This receptor could not be identified on nitrocellulose blots probed with ^{125}I-collagen. It is unclear why, in both of these cases, the solubilized receptor does not inhibit binding of ^{125}I-collagen to bacteria. However, in the case of *S. aureus*, a method was designed to identify and quantify the solubilized receptor on the basis of its ability to neutralize the inhibitory effect of antireceptor antibodies on binding of *S. aureus* to collagen (35).

The results of this study suggest that some strains of *S. mutans* contain a specific component that recognizes collagen type I and that these strains can bind to collagen in solution as well as adhere to collagen immobilized on artificial surfaces or as a constituent of dentin. The facts that (i) only strains capable of binding collagen could adhere to dentin and (ii) adhesion of *S. mutans* to dentin was inhibited only by collagen peptides suggest that recognition of collagen may be a crucial factor in the adherence of streptococci to root surfaces in the oral cavity. Collagen-binding strains of *S. mutans* may be specifically targeted to root surfaces. This hypothesis may be tested by comparing the percentage of collagen-binding *S. mutans* strains isolated from healthy and carious root surfaces with the percentage of strains isolated from other sites in the oral cavity. Ultrastructural studies on bacterial invasion and tissue breakdown in human root surface caries support the hypothesis that collagen binding not only plays a role in the tropism of *S. mutans* for dentin but also may be important in the pathogenesis of root caries. These studies show bacteria (almost exclusively gram-positive cocci) aligned along collagen fibers (14, 29). These fibers retain the original cross-banding, which suggests that intact collagen may serve as an adhesion substratum for these bacteria. If the interaction of *S. mutans* with dentin collagen is important in the etiology and pathogenesis of root surface caries, then an understanding of this interaction should allow us to design new and more effective strategies for prevention and treatment of this disease.

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