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Surface Fibrils (Fimbriae) of *Actinomyces viscosus* T14V

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Surface antigens of *Actinomyces viscosus* T14V were released from cell walls by digestion with lysozyme. These were separated by ion-exchange and gel filtration chromatography into fractions rich in carbohydrate or protein. The former contained a polysaccharide high in 6-deoxytalose, along with a peptide fragment from the cell wall. In the protein-rich fractions, material of high molecular weight was present, which contained some carbohydrate and up to 14.3% nitrogen. Aspartic acid, threonine, glutamic acid, lysine, alanine, and glycine were detected in these fractions, along with smaller amounts of 10 other amino acids. Most of the alanine was present as the L isomer and thus was not from peptidoglycan. Electron microscopy of the high-molecular-weight material revealed long fibrils, 3.5 to 4.5 nm in diameter, which resembled those seen on bacterial cells. V-specific antiserum, prepared by absorbing anti-*A. viscosus* T14V serum with cell walls of the avirulent strain (*A. viscosus* T14AV), did not react with the 6-deoxytalose polysaccharide but reacted well with isolated fibrils, and this was not inhibited by 6-deoxytalose.

The ability of *Actinomyces viscosus* and the closely related *A. naeslundii* to inhabit the mouth may require specialized cell surface structures which promote adherence to other bacteria in plaque, to teeth, and possibly to epithelial surfaces. Girard and Jacius (12) proposed that adherence of these bacteria was mediated by fine fibrils which they observed in electron micrographs. This is supported by recent studies which favor a role for the fibrils in coaggregation of *A. viscosus* with *Streptococcus sanguis* (21) and in adherence of *A. naeslundii* to epithelial cells (10). Moreover, immunoelectron microscopy performed on samples of intact human plaque revealed bacteria with fibrils that were antigenically similar to those on cultured *A. viscosus* T14 cells (4). Thus, the fibrils may be functionally similar to fimbriae or pili which occur on many gram-negative bacteria (1, 7, 8, 23) and certain gram-positive species (14, 16, 17, 28).

Hammond and co-workers (13), using antiserum against *A. viscosus* T14 virulent (V) strain, found a virulence-associated antigen (V-antigen) which was seen as an arc of precipitation in the immunoelectrophoretic pattern of cell wall antigens from *A. viscosus* T14V but not from an avirulent (AV) mutant strain, *A. viscosus* T14AV. Only the T14V strain causes periodontal disease after implantation into germfree rats (S. Socransky, personal communication). The indirect peroxidase-labeled antibody technique showed that a V-specific antiserum, prepared by absorbing anti-T14V serum with T14AV cell walls, reacted with the cell surface fibrils (4). These were more abundant on isolated cell walls from T14V than on those from T14AV but were observed on intact bacteria of both strains. Thus, the presence of fibrils and V-antigen appeared to represent quantitative rather than qualitative differences between these strains. The fibrils have not been chemically characterized; however, the V-antigen was reported (13) to be a cell wall polysaccharide containing the unusual sugar 6-deoxytalose (6-DOT).

In this communication we describe the isolation and partial characterization of fibrils from T14V cell walls. Like fimbriae or pili, the fibrils had a high content of amino acids. A V-specific antiserum reacted well with isolated fibrils but not with the cell wall polysaccharide high in 6-DOT.

MATERIALS AND METHODS

Bacteria. *A. viscosus* T14V and T14AV were kindly provided by B. F. Hammond of the University of Pennsylvania School of Dental Medicine, Philadelphia. Cells were grown to stationary phase in Trypticase-yeast extract supplemented with 0.1% glucose (4, 24).

Preparations of cell walls. The procedures used to isolate cell walls and to establish their purity by

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electron microscopy have been described previously (4). Bacterial cells harvested from 36 liters of media and disrupted with glass beads yielded approximately 5 g of dry cell walls.

Lysozyme digestion of cell walls. Lyophilized cell walls (4.5 g) were digested for 3 days at 37°C with 490 mg of egg white lysozyme (grade I; Sigma Chemical Co., St. Louis, Mo.) in 270 ml of 0.1 M ammonium acetate buffer, pH 6.25, containing 0.1% sodium azide and a few drops of toluene to prevent contamination. Undigested cell walls were collected by centrifugation, and these were redigested for 3 days with 220 mg of lysozyme in 100 ml of buffer. The digestion was lysozyme dependent since antigens were not released from cell walls incubated in buffer alone.

Soluble material from both digestions was pooled, filtered through a 0.45-μm membrane (Millipore Corp., Bedford, Mass.), dialyzed in the cold against several 4-liter changes of water, and prepared for ion-exchange chromatography by adjusting the conductivity to 0.11 mS and the pH to 8.3 with 1 M tris(hydroxymethyl)aminomethane-chloride. The insoluble residue was washed with water and lyophilized (weight, 2.2 g). Thus, lysozyme digestion solubilized approximately one-half of the starting weight of the cell walls. In gel diffusion experiments the soluble material was identical to that obtained earlier (4) from a cell wall preparation which was 90% solubilized by lysozyme.

Column chromatography. The lysozyme digest was applied to a column (2.5-cm diameter by 30 cm) of diethylaminoethyl-cellulose (DE-52; Whatman, Clifton, N.J.) equilibrated with 0.01 M tris(hydroxymethyl)aminomethane, pH 8.3. The column was washed with this buffer until lysozyme was removed, and then eluted with a 1.4-liter linear gradient of 0 to 0.3 M potassium chloride in 0.01 M tris(hydroxymethyl)aminomethane buffer to fractionate the cell wall antigens. Three peaks, designated fractions I, II, and III, were obtained. Each fraction was concentrated above an Amicon PM-10 membrane, dialyzed against phosphate-buffered saline (0.15 M sodium chloride, 0.01 M phosphate, pH 7.2), and applied to a column (2.5-cm diameter by 97 cm) of Agarose A-50m (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with phosphate-buffered saline. Dextran 2000 (Sigma Chemical Co.) and glucose were used to calibrate the column for its void and total volumes, respectively. Fraction I eluted near the total volume of the column and was recovered. Fractions II and III were separated into high (H)- and low (L)-molecular-weight fractions which were designated IIH, IIL, IIIH, and IIIL.

Column eluates were assayed for carbohydrate by the phenol-sulfuric acid reaction (6) using rhamnose as a standard, for protein by the method of Lowry et al. (19) using bovine serum albumin as a standard, for lysozyme activity with a commercial kit (Worthington Biochemicals Corp., Freehold, N.J.) which contained *Micrococcus lysodeikticus* cells, and for cell wall antigens by immunodiffusion.

Analytical methods. Amino nitrogen in hydrolyzed samples and primary amino groups in unhydrolyzed samples were measured by the ninhydrin method (26). Phosphate was determined by the method of Chen et al. (2). The anthrone reaction (15) was used to assay hexose, and values were corrected for the presence of methylpentose, utilizing 6-DOT as a standard. The Elson-Morgan method (modified) (15) was used to measure hexosamine. The conditions of hydrolysis were those given below for maximum release of reducing sugars. Methylpentose was determined by the Dische and Shettles method (15), using 6-DOT as a standard.

Reducing sugars, expressed as glucose, were determined by the Park and Johnson submicro method (15). Samples were hydrolyzed at 100°C for 2 h in 2 N HCl, which gave maximum release of reducing sugars. With pure sugars, experimentally determined values of the relative reducing power per mole were as follows: glucose, 1.00; glucosamine, 1.10; rhamnose, 0.66; and 6-DOT, 0.55. Values after hydrolysis were: glucose, 1.00; glucosamine, 1.10; rhamnose, 0.47; and 6-DOT, 0.29. Thus, the methylpentoses had less reducing power than glucose and also were partially destroyed during hydrolysis.

Amino acid analysis. Amino acid analyses were performed in the laboratory of John M. Stewart, Department of Biochemistry, University of Colorado Medical Center, Denver. Samples (approximately 1 mg of amino acids) were hydrolyzed in 6 N HCl (2 ml) at 110°C for 22 h in nitrogen-flushed, sealed tubes. Phenol and mercaptoethanol were present during hydrolysis as protecting reagents (11) for tyrosine and tryptophan, respectively. Hydrolysates were analyzed on a Beckman model 120 analyzer.

The procedure of Larson et al. (18) was used for determination of D-amino acids in the presence of their L isomers. Samples (approximately 1 mg of amino acids) containing an internal standard (DL-norleucine) were hydrolyzed in 6 N HCl (2 ml) at 110°C for 22 h and then divided into equal portions which were evaporated to dryness. One-half was treated for 4 h with 250 μg of crystalline D-amino acid oxidase (Sigma Chemical Co.) and 250 μg of catalase (2× crystallized; Sigma Chemical Co.); an outside source of oxygen was not supplied. Both enzymes were dialyzed initially against 0.02 M sodium pyrophosphate buffer, pH 8.3 (18). The enzyme-treated sample was deproteinated (18) before amino acid analysis. Several DL-amino acids were used as controls.

Purification of 6-DOT. 6-DOT was purified from a crude fraction of *A. viscosus* T14V cell walls as described by MacLennan (20). Lyophilized cell walls (4 g) were hydrolyzed in 1 N H_2SO_4 for 2 h at 100°C, and the hydrolysate was neutralized with barium hydroxide and deionized by several passages through a column of Amberlite MB-3 (Mallinckrodt, Inc., St. Louis, Mo.). Neutral sugars were collected and separated by descending chromatography with Whatman 3MM paper and butanol–pyridine–water solvent (10:3:3). The most prominent and the fastest-moving component was 6-DOT. It was located by staining thin guide strips of each paper, and the material extracted with water from these regions of the paper was rechromatographed with a different solvent (ethyl acetate–pyridine–water, 30:10:11). The 6-DOT was purified further by ethanol elution from a charcoal-celite column (15). The final yield was 72 mg.

The purified sample was compared with chemically synthesized 6-deoxy-L-talose kindly provided by Othmar Gabriel, Department of Biochemistry, Georgetown University, Washington, D.C., and the two were indistinguishable by paper chromatography in the

above-described solvent systems. In good agreement with previous data (20), the purified sample gave a cysteine-sulfuric acid reaction (15) which was 1.45 times as intense as that of rhamnose.

Rabbit antisera. Antiserum R3-6 was prepared by multiple injections of *A. viscosus* T14V cells, and the V-specific antiserum was obtained by absorbing antiserum R3-6 with cell walls of *A. viscosus* T14AV (4).

Immunological methods. Immunodiffusion experiments were performed by the plastic template technique as described previously (4, 5). Quantitative precipitin tests with the V-specific antiserum were performed by a microtechnique (3, 15), and washed precipitates were analyzed for nitrogen. 6-DOT was tested as a hapten in the quantitative inhibition of precipitation assay (3, 15).

Antigen labeling with trinitrobenzenesulfonic acid. Trinitrophenyl (TNP) derivatives of the antigen fractions from T14V cell walls were prepared by the procedure of Habeeb, as described by Means and Feeney (22). Trinitrobenzenesulfonic acid (2 mg) was added to 1.5-mg samples of each fraction in 0.16 M $NaHCO_3$ (total volume, 6 ml). After reaction with trinitrobenzenesulfonic acid for 150 min at 37°C in the dark, the samples were dialyzed in the cold against 0.1 M $NaHCO_3$, followed by dialysis against water. The extent of TNP labeling was estimated by absorbance measurements at 345 nm, using a molar extinction coefficient (1-cm path length) of 14,000 for the TNP group (22).

Electron microscopy. Whole bacterial cells and material from antigen fractions IIIL and IIIH were negatively stained and examined by standard techniques. A droplet of the specimen (50 μg/ml for the fractions) was placed on a collodion-coated grid and allowed to adsorb for 30 to 60 s. The grids were rinsed with a 0.5% solution of sodium phosphotungstate, pH 6.8, blotted to remove excess fluid, dried rapidly, and immediately examined and photographed. Several variations were employed. The sample was adsorbed to the collodion film, rinsed with saline, and then fixed for 30 s with either 2% buffered glutaraldehyde or osmium tetroxide vapor before treatment with phosphotungstate. In all instances the images of the specimen were similar.

Antigen fraction IIIH was fixed at room temperature for 2 h with 0.5% tannic acid and 0.5% glutaraldehyde buffered at pH 7.3 with 0.1 M sodium cacodylate. The insoluble fixed material was dehydrated, embedded in Epon, sectioned, and examined as described previously (4). Fraction IIIH (11 μg) and 25 μl of the V-specific antiserum (diluted 1:4) were mixed in a total volume of 0.2 ml to form an immune precipitate which was slightly on the antibody excess side of equivalence. This was washed twice with cold saline (0.4 ml per wash) and then fixed and processed for electron microscopy as outlined above. Additional staining of sections with 1% uranal acetate, lead citrate (25), or 0.5% phosphotungstic acid buffered to pH 6.8 with NaOH did not alter the appearances of the specimens significantly.

RESULTS

Fractionation of cell wall antigens. The lysozyme digest of T14V cell walls was applied to a column of diethylaminoethyl-cellulose. Elution with starting buffer removed virtually all of the lysozyme and only a trace of fraction I, as judged by immunodiffusion experiments (data not shown). Cell wall antigens were eluted with a linear gradient of KCl, and three peaks appeared (Fig. 1). Fraction I contained more carbohydrate than protein, and fractions II and III were mainly protein. Assays showed low levels of lysozyme activity throughout the KCl gradient, but this was present at a concentration of not more than 5 μg/ml and was ignored subsequently. Immunodiffusion with antiserum against *A. viscosus* T14V (Fig. 2A) and with the V-specific antiserum (Fig. 2B) demonstrated the V-antigen in fractions II and III but not in fraction I.

To further isolate the V-antigen, the three fractions were chromatographed on Agarose A-50m (exclusion limit, 50×10^6 daltons for globular molecules) (Fig. 3). The carbohydrate-containing fraction emerged near the total volume of the column and was pooled to recover fraction I (Fig. 3A). Each protein-rich fraction was separated into material of very high and of lower molecular weight (Fig. 3B and C). Four fractions (IIH, IIL, IIIH, and IIIL) were obtained, and each reacted with the V-specific antiserum (Fig. 4A, well B and Fig. 4B, well B). Two arcs of precipitation were noted with the high-molecular-weight fractions, suggesting the presence of more than a single V-antigen. These were separated well with fraction IIIH but not with fraction IIH. Antiserum against *A. viscosus* T14V cells (Fig. 4A, well A and Fig. 4B, well A) reacted with additional antigens which were most ap-

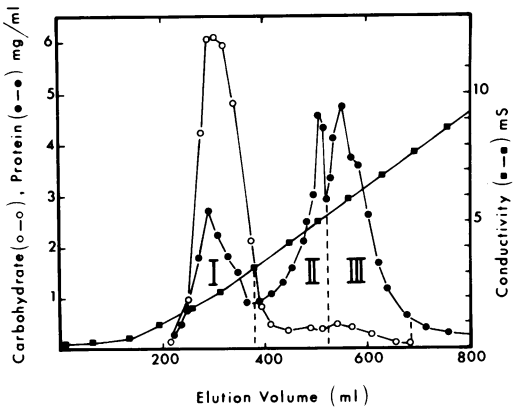

FIG. 1. *Diethylaminoethyl-cellulose ion-exchange chromatography of the lysozyme digest of A. viscosus T14V cell walls. Lysozyme was washed through the column before elution of cell wall antigens with a salt gradient. A carbohydrate-rich fraction (I) and two protein-rich fractions (II and III) were collected.*

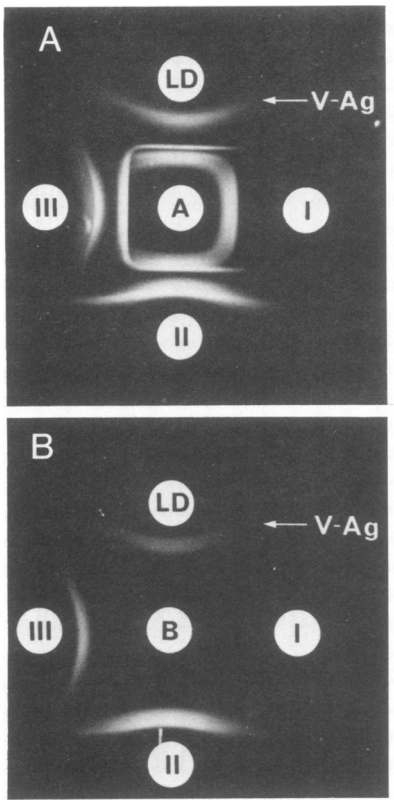

FIG. 2. *Immunodiffusion analysis of A. viscosus T14V cell wall antigens. The center wells contained antiserum R3-6 against A. viscosus T14V (A) and V-specific antiserum (B). The outer wells contained cell wall antigens in the unfractionated lysozyme digest (LD) and antigens in fractions I, II, and III from chromatography on diethylaminoethyl-cellulose. The V-antigen (V-Ag) was detected in protein-rich fractions II and III but not in carbohydrate-rich fraction I.*

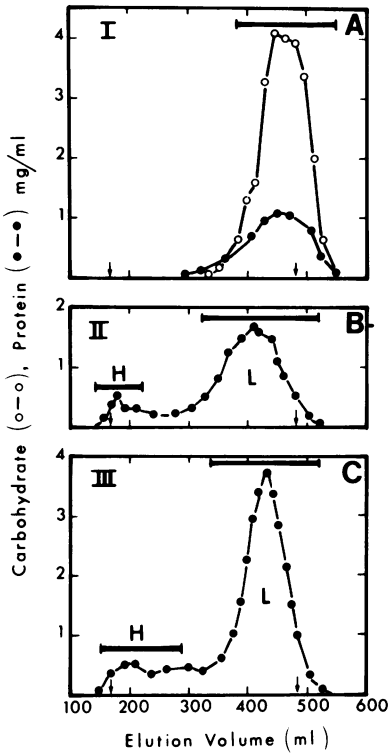

FIG. 3. *Agarose A-50m gel filtration chromatography of A. viscosus T14V cell wall antigens in fractions I (A), II (B), and III (C). Fractions II and III contained material of high (H) and of lower (L) molecular weight. Bars indicate the eluates which were pooled to give fractions I, IIL, IIH, IIIL, and IIIH. Arrows indicate the void and total volumes of the column.*

parent in the fractions of lower molecular weight.

Quantitative precipitin studies. Samples of each fraction were labeled with TNP so that precipitation of antigen by the V-specific antiserum could be measured spectrophotometrically. For every 1,000 atoms of nitrogen in antigen fractions I, IIL, IIIL, IIH, and IIIH, the number of N atoms present as free amino groups was 140, 89, 59, 68, and 64, respectively. Of these, 87, 45, 31, 33, and 25 atoms, respectively, were labeled with TNP. Labeling did not appear to destroy antigenic determinants because a reaction of identity in immunodiffusion was observed when each labeled sample was compared with its corresponding unlabeled fraction. By assuming a uniform distribution of the TNP label, it was possible to estimate the percentage of the total material in each fraction which was precipitable by antibody.

The V-specific antiserum did not precipitate a significant percentage of the total TNP-labeled material from fractions I and IIIL, because with these, removal of precipitated antigen by centrifugation left essentially all of the TNP-labeled material in the supernatant (Fig. 5A). However, with fractions IIL, IIH, and IIIH, approximately 20, 60, and 84%, respectively, of the labeled material was precipitated at equivalence (Fig. 5A). Thus, the V-specific antibodies precipitated the majority of labeled material from the fractions of high molecular weight (fractions IIH and IIIH) but failed to react with most of the labeled material in the other fractions (fractions I, IIL, and IIIL).

The five cell wall fractions were compared by their quantitative precipitin reactions with V-specific antibodies. Varying amounts of each fraction (unlabeled) were added to a constant amount of V-specific antiserum, and washed pre-

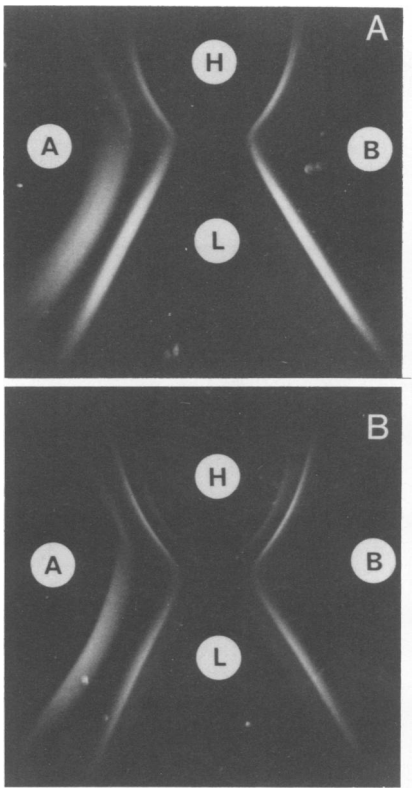

FIG. 4. *Immunodiffusion analysis of A. viscosus T14V cell wall antigens in the high (H)- and low (L)-molecular-weight fractions from Agarose A-50m chromatography. (A) Fractions IIH and IIL. (B) Fractions IIIH and IIIL. The V-antigen is shown by its precipitation with V-specific antiserum (wells B). Antiserum against A. viscosus T14V (wells A) revealed an additional cell wall antigen(s) whose presence was most obvious in the fractions of lower molecular weight.*

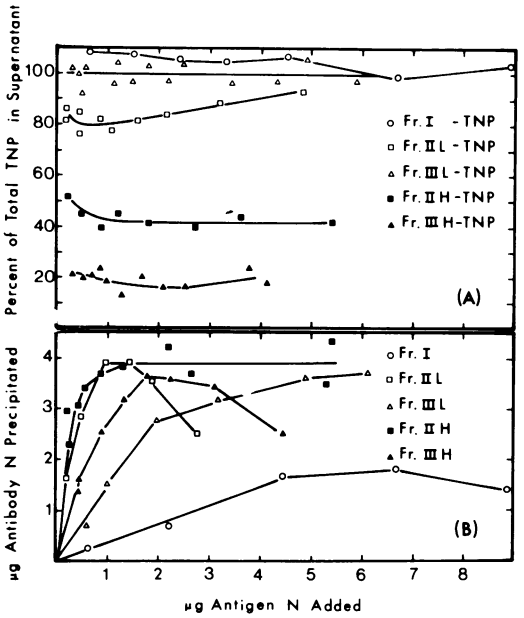

FIG. 5. *Quantitative precipitin reactions obtained with V-specific antiserum (25 μl of a 1:4 dilution). (A) V-specific antiserum and the TNP-labeled antigen fractions, showing the percentage of the total TNP label which remained in the supernatants after the removal of precipitates by centrifugation. (B) V-specific antiserum and the unlabeled antigen fractions. A total volume of 200 μl was used. The nitrogen contents of fractions IIL and IIIL were 9.7 and 11.7%, respectively; values for the other fractions are given in Table 1.*

cipitates were analyzed for nitrogen. With fractions IIL, IIH, and IIIH the amount of antigen N precipitated was estimated from the percentage of added material which precipitated (Fig. 5A), and the amount of antibody N precipitated was calculated (i.e., total N precipitated minus antigen N precipitated) and plotted against the amount of each fraction added to obtain quantitative precipitin curves (Fig. 5B). With fractions IIIL and I, the total N precipitated was taken as an approximation of the antibody N precipitated because the small contribution of antigen N to these precipitates could not be estimated from the data in Fig. 5A.

Similar amounts of antibody were precipitated from the V-specific antiserum by the four protein-rich fractions; however, the amount of each fraction required for 50% precipitation of antibody showed that fractions IIH and IIL were the most reactive, followed in order by fractions IIIH and IIIL (Fig. 5B). The high-molecular-weight fractions (fractions IIH and IIIH) contained much more precipitable material than the fractions of lower molecular weight (fractions IIL and IIIL) (Fig. 5A), but the former did not show a proportional increase in their reactivity with the V-specific antiserum (Fig. 5B). This suggested that antigens in the high-molecular-weight fractions were either poorly exposed or were associated with unreactive material which co-precipitated. Compared with the four protein-rich fractions, the carbohydrate-rich fraction (fraction I) reacted poorly, but large amounts of this material gave some precipitation with the V-specific antiserum (Fig. 5B).

Assays with 6-DOT as an inhibitor of precipitation. 6-DOT, which was reported to be a determinant of V specificity (13), was tested for its ability to inhibit precipitation of fractions IIH (1.1 μg of N) and IIIH (1.4 μg of N) with the amount of V-specific antiserum and total volume used in the quantitative precipitin assays (Fig. 5B). The highest ratios of 6-DOT added per methylpentose residue in fractions IIH and IIIH

were 7.3×10^3 and 1.8×10^4, respectively (0.165 M 6-DOT), and the inhibition was not significant (not more than 5%; data not shown).

Electron microscopy. Negatively stained preparations of the high- and low-molecular-weight fractions revealed obvious differences in their ultrastructure. High-molecular-weight fraction IIIH contained long fibrils which formed a mesh on the grid surface (Fig. 6A). Identical fibrils were observed in fraction IIH. Their diameter was between 3.5 and 4.5 nm, which was similar to the diameter of fibrils on whole bacterial cells (Fig. 6B). Images in the fractions of lower molecular weight (fractions IIL and IIIL) were difficult to interpret, but many small structures (3.5 to 4.5 nm) were present and long fibrils were seldom seen (Fig. 6C).

Fibrils also were observed in a fixed and sectioned preparation of fraction IIIH (Fig. 7A), and evidence for the binding of V-specific antibodies to these was obtained from a washed and sectioned precipitate formed slightly on the antibody excess side of equivalence (Fig. 7B). The appearance of a mesh persisted in the precipitate; however, binding of V-specific antibodies caused a thickening of the fibrils to approximately 30 nm (Fig. 7A and B).

Chemical composition of fractions. Fraction I was high in methylpentose (Table 1); this was mostly 6-DOT, with some rhamnose as shown by paper chromatography of an acid hydrolysate. The value of 41.8% for reducing sugar on hydrolysis (Table 1) was undoubtedly low since the methylpentoses present were shown to have less reducing power than the glucose standard and also were partially destroyed during hydrolysis (see above). Glutamic acid, lysine, and alanine accounted for approximately 95% of the amino acid residues detected (Table 2), which favored the presence of a cell wall peptide. The conditions of the amino acid analysis did not permit the detection of ornithine, an additional component of the peptidoglycan from *A. viscosus* (27). The overall composition of fraction I was similar to that reported previously for the V-antigen (13).

Amino acids were the major component of the fibril-containing fractions (fractions IIH and IIIH), but some carbohydrate also was present (Table 1). The amino acid compositions of these

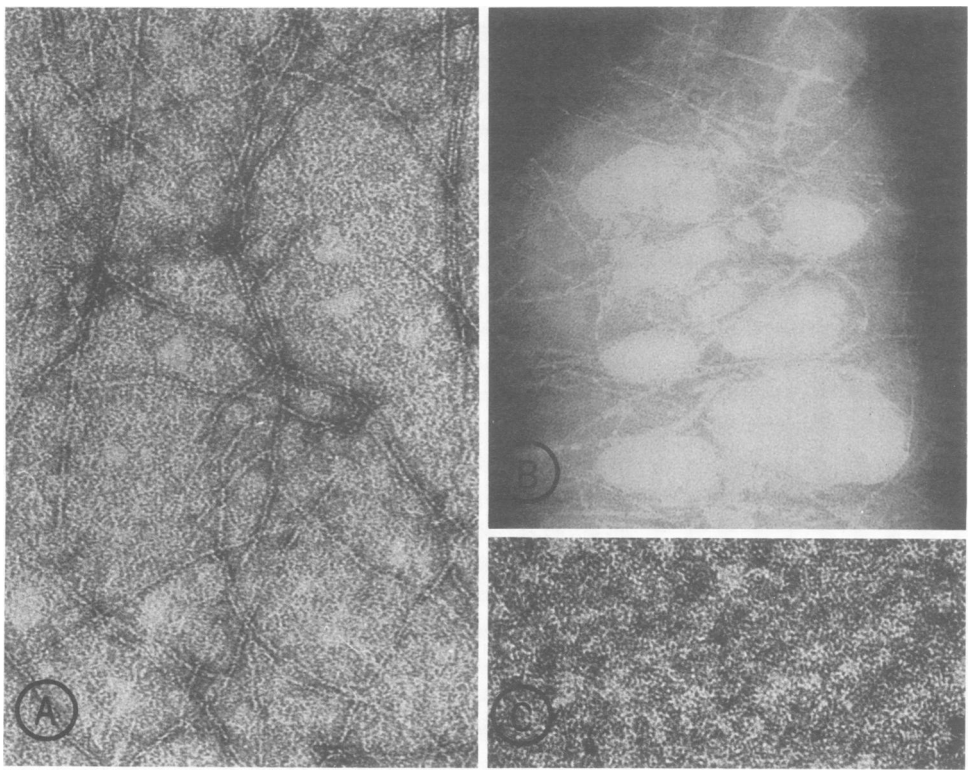

FIG. 6. *Electron micrographs of negatively stained preparations. (A) Fraction IIIH showing fibrils. ×165,000. (B) A. viscosus T14V showing fibrils on whole bacterial cells. ×145,000. (C) Fraction IIIL, which lacked fibrils. ×170,000.*

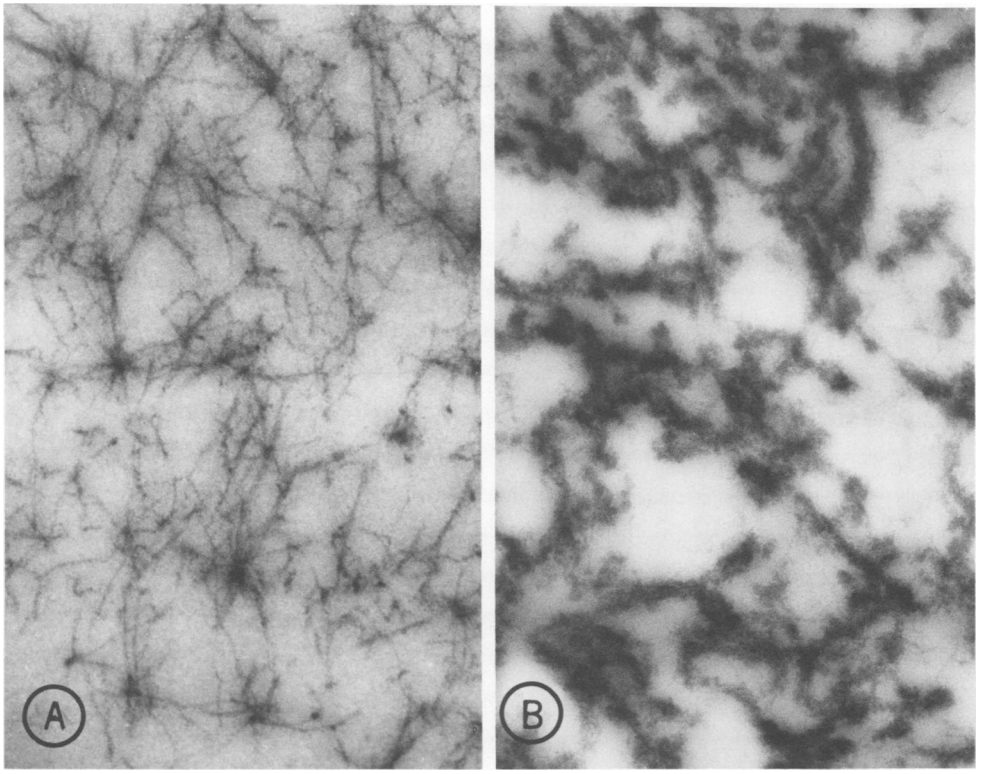

FIG. 7. *Electron micrographs of thin sections prepared from (A) fraction IIIH showing fibrils (×100,000) and (B) a washed precipitate formed with fraction IIIH and V-specific antiserum showing fibrils which are thicker than those in (A) (×60,000).*

fractions were similar and more diverse than was found with fraction I (Table 2). With fractions IIH and IIIH, polar amino acids made up approximately 65% of the total, and more acidic than basic amino acid residues were present.

The amino acids and carbohydrate found accounted for approximately 80% of the weight of fraction IIIH and somewhat less of that of fraction IIH (Table 1). With these, the nitrogen from amino acids (plus ammonia) and from hexosamines made up about 95% of the total nitrogen. Although not shown, fractions IIL and IIIL were qualitatively similar to the fibril-containing fractions but were higher in carbohydrate (32.8 and 22.3%, respectively) and lower in amino acids (44.3 and 58.5%, respectively).

Determinations for D-alanine and other D-amino acids were performed to help evaluate the contribution which amino acids from the cell wall made to the fractions which contained fibrils. As expected, fraction I contained a relatively high content of D-alanine, and only 55% of the total alanine was recovered after treatment of this material with D-amino acid oxidase (Table 3). Values for the recovery of alanine were much greater from fractions IIH and IIIH, which contained fibrils. The losses of alanine from these (14 and 10%, respectively) were of questionable significance. Losses of approximately 10% also occurred with lysine, which exists as the L isomer in peptidoglycan (27), and a 16% loss occurred for leucine in fraction IIIH. Thus, the peptide moiety of the cell wall did not make a major contribution to the fibrils.

DISCUSSION

Electron micrographs have shown fine fibrils on the surface of *A. viscosus* and *A. naeslundii* (4, 10, 12, 21). The present study provides initial data on the chemical composition of the fibrils from *A. viscosus* T14V and indicates that they are proteins or possibly glycoproteins. Thus, the terms fimbriae or pili probably should be used to designate these structures.

The fibrils of *A. viscosus* T14V and the pili from *Corynebacterium renale* strain 46 (16) have similar amino acid compositions. Pili from the latter microorganism were high in glutamic acid, aspartic acid, alanine, threonine, glycine, valine, and lysine, with lesser amounts of 11

TABLE 1. *Chemical composition of selected fractions from A. viscosus T14V cell walls*

Component	% (by wt) in:[a]		
	Fraction I	Fraction IIH	Fraction IIIH
Nitrogen	5.5	10.8	14.3
Phosphorus (PO_4)	0.6	0.2	<0.1
Reducing sugar on hydrolysis[b]	41.8	10.8	5.7
Hexose[b]	4.6	1.2	0.4
Hexosamine[c]	13.6	3.2	1.3
Methylpentose[d]	41.8	6.6	2.7
Amino acids[e]	21.7	54.3	74.4
Total percent[f]	81.7	65.3	78.8

[a] Corrections were made for the water of hydrolysis, and a total of 100% would account for the entire sample.
[b] As glucose.
[c] As glucosamine.
[d] As 6-DOT.
[e] Not corrected for losses.
[f] Includes hexose, hexosamine, methylpentose, and amino acids.

TABLE 2. *Amino acid composition of selected fractions from A. viscosus T14V cell walls*

Amino acid	Residues per 1,000 in:[a]		
	Fraction I	Fraction IIH	Fraction IIIH
Acidic		(24)[b]	(24)
Aspartic acid	7	116	139
Glutamic acid	325	124	102
Basic		(15)	(12)
Lysine	383	124	95
Arginine	0	18	20
Histidine	31	11	8
Polar uncharged		(26)	(29)
Threonine	7	112	136
Glycine	7	79	83
Serine	5	37	35
Tyrosine	0	27	33
Nonpolar		(35)	(35)
Alanine	235	120	95
Leucine	0	57	75
Valine	0	57	62
Proline	0	64	62
Isoleucine	0	33	35
Phenylalanine	0	17	19
Methionine	0	4	1

[a] Not corrected for losses.
[b] Numbers in parentheses are percentages of total amino acids.

other amino acids. Moreover, the data from *C. renale* (16) show 26% acidic, 12% basic, 26% polar uncharged, and 36% nonpolar amino acid residues. These percentages are very similar to the corresponding values given in Table 2 for fractions IIH and IIIH from *A. viscosus* T14V. Carbohydrate was not detected in the pili from

TABLE 3. *Percent recoveries of certain amino acid residues from* DL-*amino acids and cell wall fractions treated with* D-*amino acid oxidase*

Residue	% Recovery after D-amino acid oxidase treatment of:			
	DL-Amino acids	Fraction I	Fraction IIH	Fraction IIIH
Threonine	50[a]		102	96
Lysine	52	88	91	91
Alanine	57	55	86	90
Leucine	51		97	84
Valine	51		103	105
Proline	54		102	95

[a] Values were normalized by assuming a 50% loss of the internal standard, DL-norleucine.

C. renale strain 46 (16). It was present in preparations of pili from other strains (17), but this possibly was due to contamination by cell wall material. Similarly, the carbohydrate in fractions IIH and IIIH from *A. viscosus* also could represent contamination, and additional studies are needed to clarify this.

In an earlier report (13), the antigenic difference between T14V and T14AV cell walls (i.e., V-antigen) was attributed to a cell wall polysaccharide containing 6-DOT. In the present study, this was found in fraction I; however, the major antigen(s) in this fraction was not recognized by the V-specific antiserum (Fig. 2). Approximately one-half of the V-specific antibodies precipitated when large amounts of fraction I were added (Fig. 5B). This could result from material in fraction I which overlapped from fraction II (Fig. 1) and which lacked certain determinants required for complete precipitation. Although not presently described, a 6-DOT-containing polysaccharide has been isolated from T14AV cell walls, and it is antigenically identical to fraction I in immunodiffusion experiments with antisera against either T14V or T14AV whole cells. This is consistent with more extensive studies (O. Gabriel, personal communication) which have failed to reveal any differences in the biosynthesis or structure of the 6-DOT-containing polysaccharide in virulent and avirulent cells. The reaction of V-specific antiserum with isolated fimbriae was not inhibited by large amounts of 6-DOT. This suggests that 6-DOT is not an immunodominant group, but does not exclude its occurrence in oligosaccharide determinants having other immunodominant sugars. Thus, in lysozyme digests of T14V cell walls, the appearance of a V-antigen did not result from the 6-DOT-containing polysaccharide of cell wall origin. Instead, the V-specific antiserum reacted with fimbriae in fractions IIH and IIIH and recognized more than a single antigen in these

preparations (Fig. 4). A related finding is the extraction with hot 0.01 N HCl of two V-antigens from T14V cell walls (D. R. Callihan and D. C. Birdsell, Abstr. Annu. Meet. Am. Soc. Microbiol 1977, B15, p. 18). We also have observed this and found that both react with the V-specific antiserum. The nature of these antigens along with the antigenic composition of the fimbriae remain to be established.

From earlier results (4) we suggested that the V-antigen represented a quantitative rather than a qualitative difference between the virulent and avirulent strains. Recent studies (J. T. Powell and D. C. Birdsell, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, K189, p. 158) indicate that growth conditions may contribute to the expression of this difference by favoring the absence of fimbriae on the avirulent strain. Similar variations also have been found in the appearance of pili and fimbriae on gram-negative bacteria (1, 23).

Recent studies (21) favor the presence of a receptor with lectin-like properties on the surface of *A. viscosus* T14V which binds *S. sanguis* 34 to mediate coaggregation between these strains. Electron micrographs (21) suggest that coaggregation is mediated by the fimbriae. It is interesting that, unlike the T14V strain, *A. viscosus* T14AV does not coaggregate (21), but the reason for this remains to be determined. Similar to their apparent role in the coaggregation of *A. viscosus* with *S. sanguis*, the fimbriae of *A. naeslundii* may mediate interactions of this bacterium with oral streptococci (9) and with epithelial cells (10).

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