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Purification and Characterization of the Serotype *c* Antigen from *Actinobacillus actinomycetemcomitans*

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The serotype *c* antigen from *Actinobacillus actinomycetemcomitans* was purified with fractional ethanol precipitation of cell-free culture supernatant, sequential ion-exchange chromatography, and gel filtration chromatography. The preparation obtained demonstrated a single precipitin line in immunodiffusion, immunoelectrophoresis, and crossed immunoelectrophoresis when rabbit antisera to serotype *c* whole bacterial cells were used. No immunological reaction was detected with antisera to serotype *c* lipopolysaccharide, indicating that lipopolysaccharide was not present in the preparation. The serotype *c* antigen was composed of 95% carbohydrate, 2% protein, and 3.1% phosphate. Gas chromatographic analysis of the antigen obtained from growth in either complex or chemically defined media revealed that the carbohydrate constituent was composed of 84 to 90.1% mannose, 4.8 to 16% glucose, 1.9% *N*-acetylglucosamine, 1.4% fucose, and 0.2% galactose. The present data suggest that *A. actinomycetemcomitans* serotype *c* antigen is predominantly a mannose-containing carbohydrate suggestive of a mannan.

Investigations of the structure and function of microbial antigens have been useful in defining bacterial surface constituents, providing a structural basis for taxonomy, incriminating virulence factors, and illuminating the host immune response to infectious agents. A prime example is *Haemophilus influenzae* serotype *b*. The capsular polysaccharide of this species determines the serotype specificity and also is a key virulence factor by virtue of its antiphagocytic action (29).

Actinobacillus actinomycetemcomitans is a gram-negative, capnophilic, fermentative coccobacillus. This organism is suspected of being an important etiological agent in localized juvenile periodontitis by virtue of its presence in high numbers in localized juvenile periodontitis lesions (21). These patients also exhibit high levels of serum and gingival crevicular fluid antibody to this organism (7), which has been shown to elaborate a number of products which are potentially damaging to the periodontal tissues (27).

A. actinomycetemcomitans can be a serious pathogen in nonoral infections (28). It may be that of the three serotypes of *A. actinomycetemcomitans*, serotype *c* strains are particularly important in infections at nonoral sites (28). *A. actinomycetemcomitans* serotype *c* has been isolated from several cases of bacterial endocarditis, a lymphoma patient, and the type strain NCTC 9710 and strain NCTC 9709 (National Collection of Type Cultures, London, United Kingdom), which represent serotype *c*, originate from pulmonary and vertebral sites. In contrast, *A. actinomycetemcomitans* serotype *c* is not often recovered from the human oral cavity (28).

In a previous investigation, we initially determined the serotype antigens of *A. actinomycetemcomitans* to be heat-stable, high-molecular-weight, primarily carbohydrate moieties located on bacterial cell surfaces (22). In the present study, we purified and characterized the *A. actinomycetemcomitans* serotype *c* antigen.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. *A. actinomycetemcomitans* 75 (a clinical isolate) Y4 (a gift of S. S. Socransky, Forsyth Dental Center, Boston, Mass.), and 67 (a clinical isolate) were included in this study. These strains are isolates from human dental plaque and represent *A. actinomycetemcomitans* serotypes *a*, *b*, and *c*, respectively. The organisms were cultured to late logarithmic or early stationary growth phase in NIH thioglycolate liquid medium (Difco Laboratories, Detroit, Mich.) or a chemically defined medium (S. S. Socransky, personal communication) at 37°C in an anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) containing 85% N_2, 10% H_2, and 5% CO_2. Cells were harvested by centrifugation and washed three times in phosphate-buffered saline (PBS; pH 7.2).

Preparation of lipopolysaccharide. Lipopolysaccharide was extracted from *A. actinomycetemcomitans* 67 cells by the hot phenol-water technique of Westphal and Jann (25). Briefly, bacterial cells were suspended in distilled water at a concentration of 50 mg/ml and mixed with an equal volume of 90% phenol (vol/vol) at 68°C for 15 min. The mixture was cooled in ice for 10 min and centrifuged at 10,000 × *g* for 30 min, and the aqueous phase was removed. This procedure was repeated twice by the addition of equal volumes of distilled water. Aqueous phases were pooled, dialyzed against distilled water for 96 h at 4°C, and lyophilized. The lyophilized pellet was suspended in distilled water and centrifuged at 100,000 × *g* for 1 h. The supernatant was discarded, and the lipopolysaccharide pellet was lyophilized and resuspended in PBS at a concentration of 25 mg (dry weight)/ml.

Sonic extraction of bacterial cell antigens. Whole bacterial cells (100 mg [wet weight]) were placed in 15 ml of PBS in an ice bath and sonicated (Sonifier Cell Disrupter model 350; Bronson Sonic Power Co., Danbury, Conn.) at 25 W with a tapered microtip with an end diameter of 3 mm (model 419; Heat Systems Ultrasonics, Plainview, N.Y.), until more than 95% of the cells were disrupted as determined by phase-contrast microscopy. Generally, 60 min of sonication was

required. The sonic extracts were centrifuged at 12,000 × *g* for 30 min at 4°C, and the supernatants were removed, dialyzed overnight against distilled water, and lyophilized. The sonic extracts were then suspended to a protein concentration of 25 mg/ml in PBS as determined by Lowry assay (10) and stored at −20°C until used.

Antisera production. Antisera were produced in female New Zealand White rabbits weighing 4 kg to *A. actinomycetemcomitans* 75, Y4, and 67, strain 67 sonic extract, lipopolysaccharide, and the serotype *c* antigen-containing pool from ion-exchange chromatography. Bacterial cells were suspended to a concentration of 10 mg (wet weight)/ml in sterile saline, and 12 1.0-ml aliquots were administered intravenously via the marginal ear vein by the protocol of McCarty and Lancefield (12). Strain 67 bacterial sonic extract, lipopolysaccharide, and ethanol precipitate of the culture supernatant were dissolved in sterile saline to a concentration of 5, 2, and 10 mg (dry weight)/ml, respectively, and emulsified with an equal volume of Freund complete adjuvant. A total of 0.25 ml was initially injected into each hind footpad, and 0.5 ml was subsequently injected into subcutaneous sites on the back at 10-day intervals. Trial bleedings were obtained from the central ear artery, and the antibody titer was determined by serial dilution in immunodiffusion assays. Once a satisfactory antibody titer had been achieved, the rabbits were exsanguinated by cardiac puncture. Antisera were heated to 56°C for 30 min and stored in small portions at −76°C.

Rabbit antisera to *A. actinomycetemcomitans* 75, Y4, and 67 were immunoabsorbed to serotype specificity as previously described (28). Briefly, 1.0 ml of immune serum was added to 100 mg (wet weight) of whole bacterial cells from one of the heterologous serotypes. The mixture was placed in a shaker for 1 h at 37°C and then at 4°C for 12 h. After centrifugation at 16,000 × *g* for 1 h, the antisera supernatant was removed, and the absorption was repeated with bacterial cells from the other serotype. Serotype specificity of the immunoabsorbed antisera was confirmed by the absence of reactivity with bacterial strains from the other *A. actinomycetemcomitans* serotypes in immunodiffusion and indirect immunofluorescence assays.

Purification of the serotype *c* antigen. *A. actinomycetemcomitans* 67 was grown to late logarithmic or early stationary phase in NIH thioglycolate liquid medium or chemically defined medium, and the cells were harvested by centrifugation. To remove any remaining whole cells, the supernatants were passed through a 0.45-μm (pore size) filter. The cell-free culture supernatant was concentrated 10-fold by using a polysulfone filter with a 10,000-M_w porosity (Pellicon cassette system; Millipore Corp., Bedford, Mass.). Ninety-five percent ethanol equal to half the volume of the concentrate was added and incubated overnight at 4°C with continuous mixing. The precipitate was removed by centrifugation at 12,000 × *g* for 1 h, and the procedure was repeated by the addition of another volume of ethanol. The resulting ethanol precipitate (33 to 50%) was dialyzed overnight against distilled water and lyophilized. The ethanol precipitate was suspended in 0.01 M Tris-hydrochloride, pH 8.2, at a concentration of 25 mg (dry weight)/ml. A 3.0-ml sample was applied to a column (1.5 by 30 cm) of DEAE-Sephadex A-25 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) equilibrated with the same buffer. Column fractions were obtained at room temperature, monitored for nucleotides and proteins at 260 nm, and assayed for carbohydrates at 490 nm (4). Fractions (5 ml) eluted by 0.1 M NaCl were pooled, dialyzed against distilled water, and lyophilized (Fig. 1). The serotype

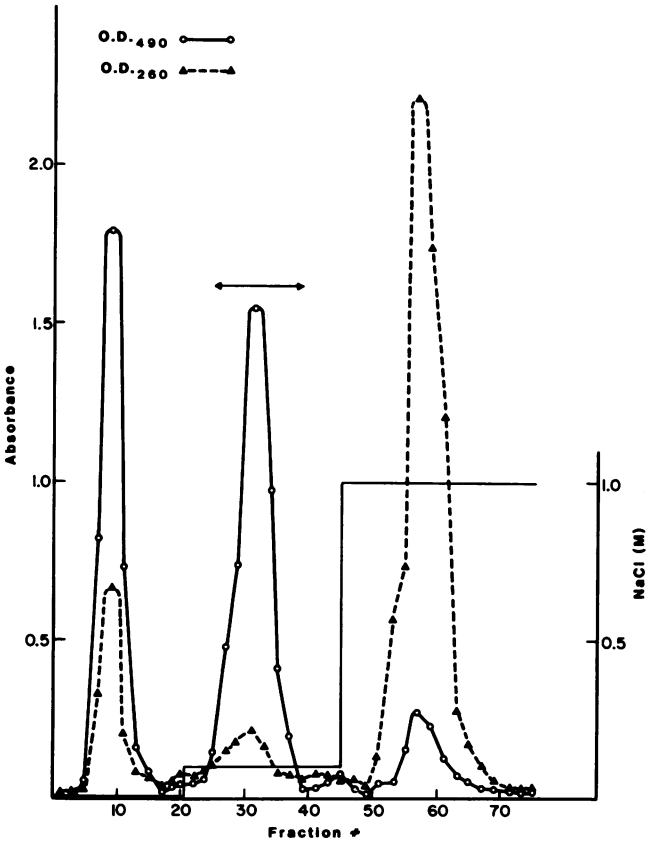

FIG. 1. Chromatography of ethanol precipitate in DEAE-Sephadex A-25. The column was equilibrated with 0.01 M Tris-hydrochloride, pH 8.2; three milliliters of sample was applied, and the column was eluted at room temperature by the isocratic addition of 0.1 M NaCl (at fraction no. 21) and 1.0 M NaCl (at fraction no. 45) to the equilibration buffer. Fractions 22 to 38 (pool II) were shown to contain serotype antigen by immunodiffusion assays with serotype-specific antisera. O.D.$_{490}$, Optical density at 490 nm.

c antigen-containing fractions (pool II) were dissolved in 0.01 M Tris-hydrochloride at 25 mg (dry weight)/ml, and 2.0 ml was applied to a column (1 by 90 cm) of Bio-Gel A5 (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with the same buffer. Fractions (3.0 ml) were collected at room temperature and assayed for nucleotides and proteins at 260 nm and for carbohydrates at 490 nm (4). Antigen-containing fractions were dialyzed overnight against distilled water and then suspended in PBS at 25 mg (dry weight)/ml.

Serological assays. Immunodiffusion was carried out in gels containing 1.2% agarose (Sea Kem; FMC Corp., Rockland, Maine) in 0.33 M Veronal buffer, pH 8.2, by the method of Ouchterlony (14).

Immunoelectrophoresis was performed in 1.0% agarose in 0.33 M Veronal buffer, pH 8.2, at 6 V/cm for 50 to 60 min (16).

Crossed immunoelectrophoresis was conducted in 1.0% agarose in 0.02 M barbital buffer, pH 8.6, on an LKB Multiphor apparatus (LKB Instruments Inc., Rockville, Md.) equipped with a water-cooling plate (2 to 4°C). The first dimension was carried out at 10 V/cm for 1 h, followed by electrophoresis at 4 V/cm in the second dimension for 12 h in 1.0% agarose containing a 1:50 or 1:100 dilution of antiserum (2).

Indirect immunofluorescence was carried out essentially as described by Mouton et al. (13). *A. actinomycetemcomitans* 75, Y4, and 67 were grown for 3 to 5 days on Trypticase soy blood agar (BBL Microbiology Systems, Cockeysville, Md.). Cells were harvested by means of a platinum loop and placed in PBS to an optical density at 540 nm of 0.7. Ten microliters of the bacterial suspension was distributed onto prewashed glass slides and gently heat fixed. Ten microliters of immunoabsorbed rabbit antiserum at working titer (the highest two-fold serial dilution still giving brilliant fluorescence of the cell envelope, generally 1:256 to 1:512) and at working titer concentrated four times (1:16 to 1:32) in PBS containing 0.05% Tween 20 was placed on bacterial smears for 10 min and then rinsed with PBS containing 0.05% Tween 20, washed in PBS, and rinsed with distilled water. The slides were then incubated with 25 μl of goat anti-rabbit immunoglobulin G conjugated with fluorescein isothiocyanate isomer I (BBL; 25 μg of fluorescein per mg of protein) and diluted to a ratio of 1:100 with PBS containing 0.05% Tween 20. The slides were rinsed and washed as before and then mounted with glycerol in PBS (2:1 [vol/vol]), pH 9.0.

A Leitz Orthoplan microscope equipped for phase-contrast illumination and incident light fluorescence was used to examine the stained bacterial smears. The light source was an Osram HBO-200 mercury lamp with a BG filter and a dichroic 495-nm interference filter in the exciting pathway with a K510 suppression filter. Fluorescence was graded from 1+ to 4+, with grades 3+ and 4+ considered to be serologically positive reactions.

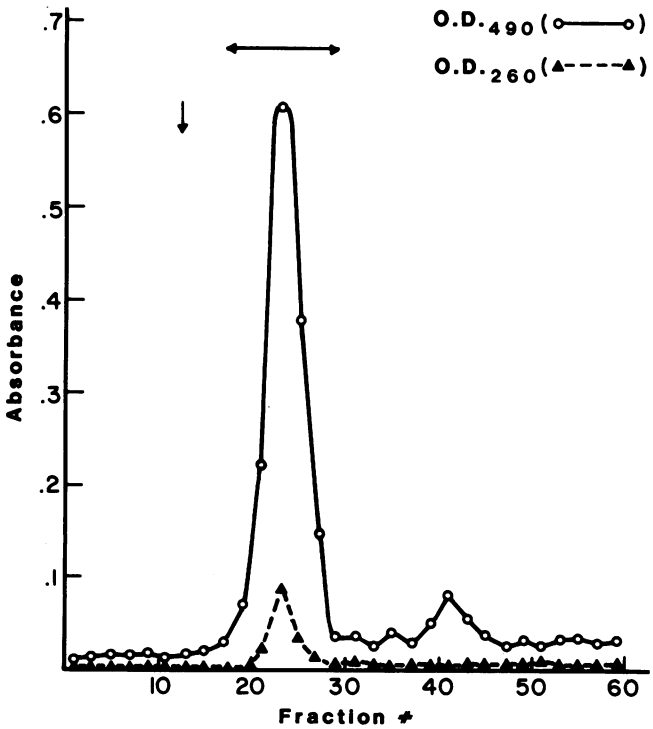

FIG. 2. Chromatography of pool II (see Fig. 1). Lyophilized pool II fractions were suspended in 0.01 M Tris-hydrochloride buffer, pH 8.2, at 25 mg (dry weight)/ml, and 2.0 ml was applied to a column of Bio-Gel A5 equilibrated and eluted with the same buffer at room temperature. A single antigen-containing peak was obtained (horizontal arrow), as determined by immunodiffusion reactions, with serotype-specific antisera. The vertical arrow indicates the void volume. O.D.$_{490}$, Optical density at 490 nm.

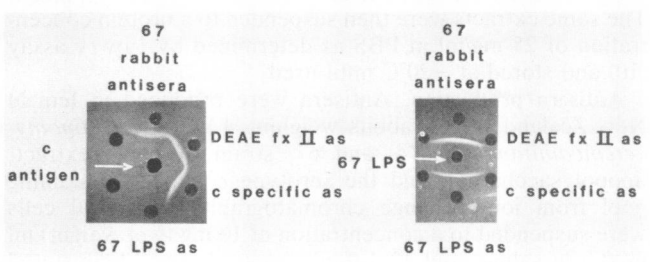

FIG. 3. Immunological characterization of *A. actinomycetemcomitans* serotype *c* antigen. Left, immunodiffusion reactions with 30 μl each of rabbit antiserum to *A. actinomycetemcomitans* 67 (serotype *c* representative) and pool II (DEAE fx II as) from DEAE column chromatography, *A. actinomycetemcomitans* serotype *c*-specific rabbit antiserum (*c* specific as), and antiserum to strain 67 lipopolysaccharide (67 LPS as). The center well contained 30 μl of *A. actinomycetemcomitans* serotype *c* antigen at a concentration of 25 mg (dry weight)/ml in PBS. Right, as above with 30 μl of strain 67 lipopolysaccharide (67 LPS) at a concentration of 25 mg (dry weight)/ml in PBS in the center well.

To determine the relationship between the antigen involved in the precipitin reactions and the antigen in the fluorescent reactions, two 10-μl aliquots of serotype *c*-specific antiserum were absorbed with 1 mg of either dextran or purified serotype *c* antigen in a shaker at 37°C for 1 h and then at 4°C for 12 h. The mixture was centrifuged at 16,000 × *g* for 60 min, the antiserum supernatant was removed, and the absorption was repeated. Twofold serial dilutions of the absorbed antiserum were prepared and examined in immunofluorescent assays by using unabsorbed serotype *c*-specific antiserum as a control.

Quantitative chemical analysis. Total sugars were assayed by the phenol-sulfuric acid method (4), and neutral sugars were assayed by the anthrone reaction (17). Phosphorus was determined by the method of Bartlett (3), and protein was measured by the procedure of Lowry et al. (10). Ketodeoxyoctonate was analyzed by using the microassay of Karkhanis et al. (8).

Quantitative determination of individual sugars was performed by two separate procedures. First, samples were methanolyzed to their methyl glycosides by treatment with 0.5N hydrogen chloride-methanol at 78 to 80°C for 20 h. The hydrogen chloride-methanol was removed under vacuum, and the residues were silylated by the addition of Tri Sil Z (Pierce Chemical Co., Rockford, Ill.). Sugars were then identified as their methyl ethers on a Varian gas chromatography model 2740 by using an OV-101 column (Alltech Associates, Inc., Applied Science Div., State College, Pa.) with a temperature progression from 140 to 200°C at 2°C/min.

Alternatively, samples were hydrolyzed in 2 N HCl for 6 h at 100°C. Hydrolysates were then passed through coupled columns of Dowex 50-X4 H^+ (200-400 mesh) and Dowex 1-X8 formate (200-400 mesh). Hexosamines were eluted from the Dowex 50 columns with 2 M HCl and then quantitated on a Beckman 120C amino acid analyzer. Neutral sugars in the

effluent water wash were converted to their alditol acetate derivatives as described by Kessler et al. (9). The alditol-acetates were then analyzed on a Hewlett-Packard model 5992 gas chromatograph-mass spectrometer, using a glass column (0.2 by 220 cm, packed with 3% OV-225) and run isothermally at 180°C with helium as the carrier gas (25 ml/min). The various peaks were identified by their retention times with respect to tetra-O-methylglucitol.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Purified serotype *c* antigen (200 μg) was incubated for 2 h at 37°C in 0.1 M sodium phosphate buffer (pH 7.0) with 1% sodium dodecyl sulfate in the presence of 1% 2-mercaptoethanol (24). The sample was then applied to duplicate 4% acrylamide gels and electrophoresed at 8 mA per gel. Gels were then stained with either Coomassie blue or periodic acid-Schiff reagent (6).

RESULTS

Preparation of *A. actinomycetemcomitans* serotype *c* antigen. Initial studies revealed that *A. actinomycetemcomitans* serotype *c* antigen is shed into the bacterial culture medium during growth, can be efficiently recovered from a 33 to 50% ethanol precipitate of culture supernatant, and is heat stable (80°C, 30 min). The latter finding suggested that the serotype *c* antigen may be primarily carbohydrate.

The fractionation of the 33 to 50% ethanol precipitate on DEAE-Sephadex A-25 is shown in Fig. 1. The addition of 0.1 M NaCl to the equilibration buffer eluted a major carbohydrate peak which contained the serotype *c* antigen (pool II). Subsequent chromatography of pool II on columns of Bio-Gel A5 separated remaining protein from the serotype *c* antigen (Fig. 2). A single peak eluted within the included volume. This peak material reacted immunologically with serotype *c*-specific antisera. Additional gel filtration on columns of Sephadex G-100 resulted in the elution of serotype *c* antigen at the void volume, indicating a molecular weight of more than 100,000.

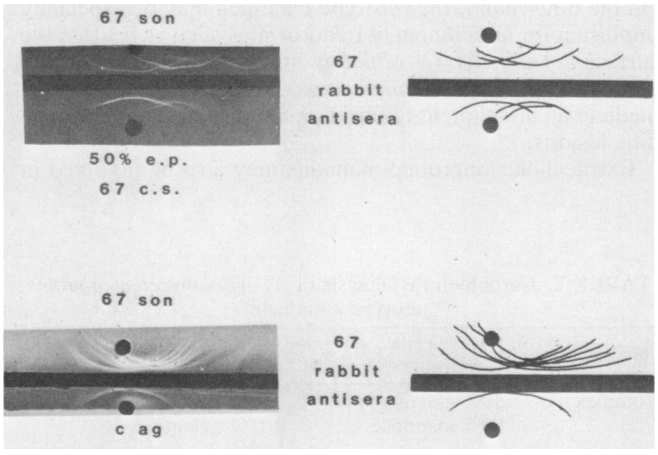

FIG. 4. Immunoelectrophoretic reactions of the serotype *c* antigen. Top, 30 μl each of *A. actinomycetemcomitans* 67 bacterial sonic extract (67 son) at a protein concentration of 25 mg/ml in PBS and the 33 to 50% ethanol precipitate (50% e.p.) from strain 67 cell-free culture supernatant (67 c.s.) at a concentration of 25 mg (dry weight)/ml in PBS was electrophoresed at 6 V/cm for 50 to 60 min and reacted with 80 μl of rabbit antiserum to strain 67. Bottom, 30 μl each of *A. actinomycetemcomitans* 67 bacterial sonic extract and the serotype *c* antigen (c ag) (25 mg [dry weight]/ml) were electrophoresed and reacted with 80 μl of rabbit antiserum to strain 67.

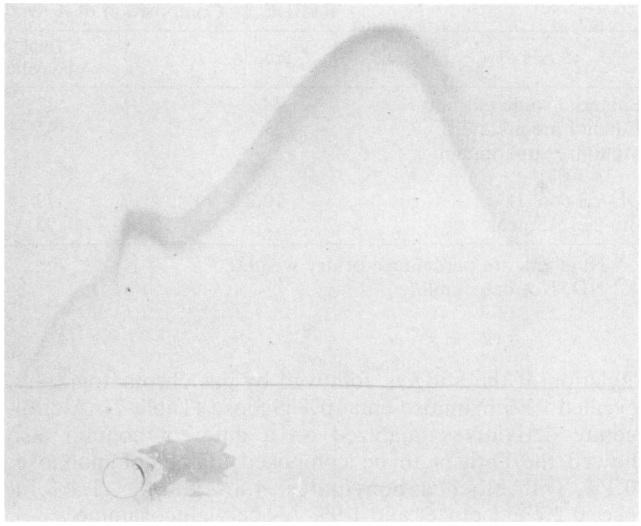

FIG. 5. Crossed immunoelectrophoresis of the serotype *c* antigen. Forty microliters of serotype *c* antigen at a concentration of 25 mg (dry weight)/ml in PBS was electrophoresed for 1 h at 10 V/cm in the first dimension and at 4 V/cm for 12 h in the second dimension in 1.0% agarose containing a 1:50 dilution of rabbit antiserum to strain 67 bacterial cells.

Immunological characterization of *A. actinomycetemcomitans* serotype *c* antigen. In immunodiffusion assays, the purified serotype *c* antigen produced a single precipitin line of complete identity with rabbit antisera to *A. actinomycetemcomitans* 67, serotype *c*-specific rabbit antisera, and rabbit antisera produced against pool II but not to rabbit antisera against strain 67 lipopolysaccharide (Fig. 3). In Fig. 3 it can also be seen that strain 67 lipopolysaccharide formed a precipitin band with rabbit antisera to strain 67 whole cells and with antisera to strain 67 lipopolysaccharide but not to either serotype *c*-specific antisera or antisera to DEAE fraction II. The absence of lipopolysaccharide was confirmed by the absence of keto-deoxyoctonate in pool II.

Immunoelectrophoresis and crossed immunoelectrophoresis showed that when assayed against strain 67 whole-cell antisera, purified serotype *c* antigen gave a single precipitin band (Fig. 4 and 5), strain 67 whole-cell sonic extract gave numerous bands, and the 33 to 50% ethanol fraction of cell-free culture supernatant gave two bands (Fig. 4).

Immunofluorescence assays indicated that purified serotype *c* antigen was involved in both precipitin assays and immunofluorescent assays of strain 67 bacterial cells. Absorption of serotype *c*-specific antisera with purified serotype *c* antigen effectively eliminated the immunofluorescence reaction with strain 67 bacterial cells (positive immunofluorescence at 1:4 dilution), whereas absorption with dextran had little effect (positive immunofluorescence at a 1:64 dilution compared with a 1:128 dilution for serotype *c*-specific control antiserum).

Chemical analysis of serotype *c* antigen. The purified serotype *c* antigen from *A. actinomycetemcomitans* grown in thioglycolate broth contained 2% protein, 95% total carbohydrate, of which 88.9% was hexose, no detectable keto-deoxyoctonate, and 3.1% phosphate (Table 1).

Carbohydrate analysis was performed by two different hydrolysis conditions and analytical procedures (Table 2).

TABLE 1. Composition of *A. actinomycetemcomitans* serotype *c* antigen[a]

Strain 67	Protein	Total carbohydrate	Hexose	Phosphate	Keto-deoxyoctonate
Bacterial sonic extract	48	12	7.3	2.4	0.66
Ethanol precipitate of culture supernatant (33-50%)	23	45	34	1.2	0.14
DEAE pool II	10.2	79	58	2.8	ND[b]
Bio-Gel A5 peak	2	95	88.9	3.1	ND

[a] Numbers are percentage of dry weight.
[b] ND, Not detectable.

Silylation of the antigen, followed by gas chromatography, revealed 90% mannose and 10% glucose (Table 2). Alditol-acetate derivatives analyzed on a different column also showed the antigen to be composed mainly of mannose, 90.1%, with other carbohydrates: 4.8% glucose, 1.4% fucose, 0.2% galactose, and 1.9% *N*-acetylglucosamine.

Purification and chemical characterization of serotype *c* antigen was repeated with *A. actinomycetemcomitans* 67 cells grown in a chemically defined medium. After 50% ethanol precipitation of the cell-free culture supernatant, the dialyzed and concentrated material was placed on a DEAE-Sephadex A-25 column, using conditions described earlier. Fraction elutions with 0.1 M NaCl were shown in immunodiffusion assays to contain the serotype *c* antigen. After dialysis and lyophilization, the carbohydrate content of the serotype *c* antigen was determined. As found for the complex medium, the antigen isolated from chemically defined medium also contained mainly mannose (Table 2).

Electrophoresis. Polyacrylamide gel electrophoresis of the serotype *c* antigen purified from thioglycolate medium resulted in a single broad band which was only demonstrable by periodic acid-Schiff staining under reducing conditions. No protein components were apparent with Coomassie blue staining.

DISCUSSION

The serotype antigens of *A. actinomycetemcomitans* are cell surface components demonstrable by immunofluorescent staining (28). The high levels of serum antibodies to these antigens seen in humans infected with oral *A. actinomycetemcomitans* (28) suggest that these antigens may be important virulence factors.

The purified *A. actinomycetemcomitans* serotype *c* antigen is a high-molecular-weight substance. With serotype *c*-specific antisera, the antigen was detected by immunodiffusion in DEAE-Sephadex A-25 pool I, which was not bound to the column as well as in pool II, which was eluted by the addition of 0.1 M NaCl. In contrast to pool I, however, immunodiffusion assays of pool II revealed only a single precipitin band and therefore contained fewer contaminants. Gel filtration chromatography of pool II on Sephadex G-100 (molecular exclusion limit of 100,000) resulted in elution with the void volume, but by using Bio-Gel A5 (molecular exclusion limit of 5,000,000), a single symmetrical peak was obtained in the included volume. The high molecular weight of the serotype *c* antigen was also evident from polyacrylamide gel electrophoresis when the antigen barely permeated 7.5 and 5.0% polyacrylamide gels but formed a broad band in 4% gels.

The *A. actinomycetemcomitans* serotype *c* antigen is primarily a mannose-containing carbohydrate. The antigen was observed in polyacrylamide gels after electrophoresis and periodic acid-Schiff staining but was undetectable with Coomassie blue staining. Chemical analysis also confirmed a high carbohydrate-to-protein ratio in the antigen and furthermore showed with two different hydrolysis conditions and two different analytic techniques, that the antigen is primarily composed of mannose, suggestive of a mannose polymer such as a mannan. Other carbohydrate moieties in this antigen included fucose, galactose, glucose, and *N*-acetylglucosamine.

Mannans are commonly found in the cell wall of yeasts such as *Saccharomyces cerevisiae* and *Candida albicans* (11, 18, 26). Mannans have also been demonstrated in the gram-positive bacterium *Micrococcus lysodeikticus* (15) and recently in the gram-negative species *Pasteurella multocida* (5), an organism taxonomically related to *A. actinomycetemcomitans*, but generally they appear to be unusual cell envelope constituents of bacteria.

As a cell surface component, the serotype *c* antigen might mediate an attachment of *A. actinomycetemcomitans* to oral epithelial cells (1, 21) in a manner similar to that in which cell wall mannans enable *C. albicans* to adhere to buccal epithelial cells (18). However, since only a relatively small number of human subjects harbor oral *A. actinomycetemcomitans* serotype *c* (28), this mannan may not be as effective as serotypes *a* and *b* in mediating an attachment in oral sites. On the other hand, the serotype *c* antigen may be especially important for attachment at nonoral sites such as heart valve surfaces. Like the *C. albicans* surface mannan (11), the mannan from *A. actinomycetemcomitans* serotype *c* may mediate an attachment to fibrin-platelet matrices in endocarditis lesions.

Extracellular microbial mannans may also be involved in

TABLE 2. Carbohydrate analysis of *A. actinomycetemcomitans* serotype *c* antigen[a]

Medium	Methyl ether derivatives[b]	Alditol-acetate derivatives[c]
Complex	10% glucose 90% mannose	1.37% fucose 0.15% galactose 1.92% *N*-acetylglucosamine 4.80% glucose 90.12% mannose
Defined	16% glucose 84% mannose	ND[d]

[a] Figures are percentages of total recoverable carbohydrate.
[b] Methanolysis in 0.5 N hydrogen chloride-methanol at 78 to 80°C for 20 h.
[c] Hydrolysis in 2 N HCl at 100°C for 6 h.
[d] ND, Not determined.

postattachment events of an infection. Yeast mannans are capable of specifically inhibiting the oxidative burst of respiration and myeloperoxidase release from polymorphonuclear leukocytes without impairing chemotaxis, random motility, degranulation, or phagocytosis (26). This impairment of oxidative microbial killing by neutrophils may, together with resistance to the bactericidal effect of serum (23), permit *A. actinomycetemcomitans* to evade the host defense system. Moreover, the small amount of phosphate detected in the serotype *c* antigen may be indicative of a phosphomannan which, in other microorganisms, can inhibit fibroblast proliferation and potentiate disease processes involving connective tissue (19). Interestingly, sonic extracts of *A. actinomycetemcomitans* have been demonstrated to impair fibroblast proliferation (20).

Further studies are needed to determine the specific role of the *A. actinomycetemcomitans* serotype *c* antigen in initial colonization, invasion, and tissue destruction in oral as well as nonoral infections.

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