Isolation of a Corncob (Coaggregation) Receptor Polypeptide from *Fusobacterium nucleatum*

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Corncocks, which are distinct morphological units formed by the ordered coaggregation of a filamentous microorganism and streptococci, can be made in vitro by using oral strains of *Fusobacterium nucleatum* and *Streptococcus sanguis*. Previous studies have shown that strains of *F. nucleatum* contain one of at least two different types of corncob receptor. The objective of this study was to isolate the receptor from *F. nucleatum* ATCC 10953 as the first step in the elucidation of the molecular basis of corncob formation. The cell envelope fraction from this bacterium was treated with trypsin, delipidated with chloroform-methanol, and subjected to ion-exchange chromatography. A single polypeptide (apparent Mr, 39,500), which was eluted from the column with 0.5 M sodium chloride and extracted with dodecylmethylammonium bromide to remove contaminating lipopolysaccharide, inhibited corncob formation between strain ATCC 10953 and *S. sanguis* CC5A. Similarly derived cell fractions from either *F. nucleatum* FDC 364 or *Fusobacterium necrophorum* failed to effect coaggregation in the inhibition assay. Amino acid analysis of the polypeptide showed a moderately hydrophobic character (polarity index, 41) and 11% basic residues. Antiserum made against the purified polypeptide agglutinated *Fusobacterium nucleatum* ATCC 10953, neutralized the ability of this bacterium to form corncocks, and agglutinated whole cells of *S. sanguis* CC5A that were precoated with the receptor polypeptide. The identification and isolation of this receptor should greatly enhance our ability to define some of the complex intergeneric coaggregation mechanisms that are thought to occur in the human oral cavity.

Corncob formations are specific bacterial coaggregations that are found at the maturing surface of human dental plaque. They are composed of a central rod-shaped or filamentous bacterium surrounded by bound streptococci, hence, the designation "corncocks." These coaggregates are highly ordered structures and are usually composed of the gram-positive pair *Bacterionema matruchoti*--*Streptococcus sanguis* (15) or the gram-negative--gram-positive pair *Fusobacterium nucleatum*--*S. sanguis* (17). *S. sanguis* is one of the first microorganisms to colonize the surface of the tooth and, as such, is a predominant component of dental plaque (27). Anaerobic, gram-negative bacteria such as *F. nucleatum*, on the other hand, generally bind poorly to enamel or the salivary pellicle and are more intimately associated with soft tissue destruction, contributing to gingivitis or other forms of periodontal disease (22, 25, 28, 32). Thus, one can envision coaggregations between *S. sanguis* and gram-negative bacteria as one mechanism for the growth of human dental plaque from a supragingival to a subgingival location.

Corncob-forming bacteria have been isolated from in vivo aggregates by micromanipulation (24) and have been used to study the process of coaggregation in vitro (17, 25, 30). We have been studying the process of coaggregation of *F. nucleatum* and *S. sanguis* and have reported on the kinetics (20) and strain specificity (7; J. Kaufman and J. M. DiRienzo, Oral Microbiol. Immunal, in press) of the interaction. In these studies several lines of evidence were presented indicating that strains of *F. nucleatum* can be divided into at least two groups on the basis of their coaggregation properties. The evidence for two classes of corncob receptors was based on strain differences in relation to (i) kinetics of corncob formation, (ii) inhibition of corncob formation by lipoteichoic acid, (iii) effects of acridine orange on the ability of the bacterium to form corncocks, and (iv) inhibition of corncob formation by specific bacterial cell fractions. In this report we describe the isolation and initial characterization of the coaggregation receptor from *F. nucleatum* ATCC 10953.

MATERIALS AND METHODS

Bacterial strains and culturing conditions. *F. nucleatum* ATCC 10953 and FDC 364, *Fusobacterium necrophorum* ATCC 9432, and *S. sanguis* CC5A were used in this study and have been described previously (20). The fusobacteria were grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.2% yeast extract (Difco) and 0.5 mg of L-cysteine hydrochloride per ml in an anaerobic chamber (Forma Scientific, Marietta, Ohio) in a mixed gas atmosphere containing nitrogen (80%), hydrogen (10%), and carbon dioxide (10%) for 18 to 24 h. *S. sanguis* CC5A, which was previously isolated from in vivo corncocks (24), was grown in brain heart infusion broth as standing cultures. Growth of bacteria was followed with a spectrophotometer at 600nm.

Preparation of cell fractions. Cell envelope and soluble protein fractions were isolated from the fusobacteria as reported previously (8). Briefly, cells harvested from 8-liter cultures were washed, suspended in 400 ml of 10 mM Tris hydrochloride (pH 8.0), and disrupted by sonication. Unbroken cells were removed by centrifugation at 12,100 × g (SS-34 rotor; Ivan Sorvall, Inc., Norwalk, Conn.) for 10 min, and the envelope fraction was sedimented by centrifugation at 85,000 × g (50Ti rotor; Spinco) for 60 min. The protein that remained in suspension following the high-speed centrifugation was designated the soluble protein fraction and contained both cytoplasmic and periplasmic proteins.

Lipopolysaccharide (LPS) was extracted from whole cells of *F. nucleatum* ATCC 10953 by the method of Westphal and Jann (34). Cells (1 liter of culture) were grown for 24 h, harvested, washed, and extracted with 45% aqueous phenol.
at 56°C for 30 min. The mixture was cooled in an ice bath and centrifuged at 1,085 × g (SS-34 rotor; Sorvall) for 15 min to separate the aqueous and phenol phases. The aqueous layer was removed, and the phenol layer was extracted with an equal volume of deionized water. The combined water phases were dialyzed against 0.05 M magnesium chloride at 5°C. The dialysate was treated with 50 µg of DNase and RNase (Sigma Chemical Co., St. Louis, Mo.) per ml at 37°C for 30 min. The preparation was centrifuged at 143,000 × g (50Ti rotor; Spinco) for 2 h, and the resulting pellet was washed once with 0.05 M magnesium chloride. The final pelleted material was assayed for total neutral sugar by the phenol-sulfuric acid assay (9) and hexosamines on the amino acid analyzer. The LPS was stored in deionized water at −20°C.

Corncob inhibition assay. A modification of the quantitative corncob assay (20) was used to measure the inhibitory activity of bacterial cell fractions during the purification of the corncob receptor. Cells of S. sanguis CC5A were labeled by the addition of 2 µCi of [methy1-3H]thymidine (25 Ci/ mmol; Amersham Corp., Arlington Heights, Ill.) per ml of culture. The cells were grown for 18 h and treated as described below. The specific activity of the labeled streptococci was approximately 5 × 10^6 cpm/2 × 10^9 cells.

For the assay, radiolabeled S. sanguis CC5A and the fusobacteria were harvested by centrifugation and washed in cold 0.15 M NaCl (1/10 the original culture volume). The cells were suspended in 0.15 M NaCl such that a 1:10 dilution of the suspension yielded an A_{490} of 0.9 (Ultrospec model 4050: LKB Instruments, Inc., Paramus, N.J.). This absorbance value was equivalent to a reading of 100 on a Klett-Summerson photometer with a blue (470-nm) filter. The cell suspensions could be stored on ice for a maximum of 5 days without the loss of corncob-forming ability.

The corncob reaction mixture consisted of 1.75 ml of 50 mM Tris hydrochloride (pH 7.5), 0.2 ml of labeled streptococci suspension (2 × 10^9 cells), and 0.05 ml of fusobacterial suspension (5 × 10^7 cells). The reactions were carried out in 5-ml polystyrene test tubes (12 by 75 mm; W. Sarstedt, Inc., Princeton, N.J.) and were incubated for 1 h at 37°C on a rocking platform mixer. The presence of corncob was quantitated by retention of the coaggregates on 5-µm-pore-size polycarbonate filters (Nuclepore Corp., Pleasanton, Calif.). To test the ability of cell fractions from F. nucleatum ATCC 10953 to inhibit corncob formation, the cell fraction was preincubated with the labeled streptococci for 1 h at 37°C, followed by the addition of whole cells of F. nucleatum. Positive inhibition resulted in the reduction of the amount of labeled streptococci retained on the filter compared with that in control tubes lacking the inhibitor. Thus, inhibition was calculated as a percentage of control corncob (streptococci and fusobacteria without inhibitor). Control values ranged from 2 × 10^9 to 3 × 10^9 streptococci bound, after correcting for background (autoagglutination of streptococci). Each concentration of inhibitor tested was run in triplicate. Cell fractions from heterologous strains were tested for their corncob-inhibitory activity in the same manner. The background radioactivity nonspecifically trapped on the filters was determined by incubating the labeled streptococci plus inhibitor at each concentration in the reaction tube, filtering the contents of the tube, and subtracting these values from the experimental results. Background values ranged from 4 × 10^7 to 10 × 10^7 cells. This autoagglutination of the streptococci was kept to a minimum by the use of specific growth and buffer conditions (20). Occasionally, when batches of streptococci formed long chains, the preparations were discarded. All cell preparations and assay results were checked microscopically.

Glucosamine, N-acetyl-d-glucosamine, and galactose (Sigma) were tested for their corncob-inhibitory activities. The sugars were preincubated, at concentrations ranging from 25 to 150 mM, with the labeled streptococci or fusobacteria as described above for the fusobacterial cell fractions. Antisera made against whole cells of F. nucleatum and the receptor polypeptide were also tested for their ability to block corncob formation. In each case the sera (dilutions ranging from 1 × 10^{-2} to 5 × 10^{-4}) were preincubated with F. nucleatum for 1 h at 37°C prior to the addition of S. sanguis. Preimmune rabbit serum was run as a control at dilutions ranging from 1 × 10^{-2} to 2.5 × 10^{-3}.

An agglutination assay was also used to determine whether the purified receptor polypeptide could bind to S. sanguis CC5A. The polypeptide (25 µg) was preincubated with the radiolabeled streptococci for 30 min at 37°C, and then anti-polypeptide serum was added in increasing amounts (dilutions of 1 × 10^{-2} to 5 × 10^{-4}). Following incubation for an additional 30 min the samples were filtered and counted. Preimmune serum was also used as a control. The results were expressed as the amount of radioactivity retained on the filters.

Isolation of the coaggregation receptor polypeptide. The cell envelope fraction was obtained from 8 liters of culture of F. nucleatum ATCC 10953 as described above. This fraction was treated with trypsin (1 mg of enzyme per 2.8 mg of protein) for 2 h at 37°C. Trypsin inhibitor was then added to a final concentration of 1 mg/1.6 mg of trypsin and the incubation was continued for an additional hour. The treated sample was centrifuged at 85,000 × g for 30 min and washed twice with deionized water. The sedimented material was delipitated by extraction with chloroform-methanol (2:1; vol/vol) in the cold for 10 min (1). The aqueous phase was concentrated by lyophilization, suspended in deionized water, and centrifuged at 85,000 × g for 30 min. The insoluble pellet was then dissolved by brief sonication in 10 mM triethylamine (pH 8.1) containing 9 M urea. The sample was exhaustively dialyzed against deionized water, and 30 µg of protein was applied to a DEAE-Sephadex column (1.5 by 70 cm; bed volume, 100 ml) equilibrated with 10 mM Tris hydrochloride (pH 8.0). The column was washed with 200 ml of buffer followed by a stepwise elution with 100 ml each of column buffer containing 0.2 and 0.5 M sodium chloride. The column effluent was monitored at A_{280}; and the protein peak that eluted with 0.5 M salt was collected, dialyzed, and concentrated by lyophilization. This material was then extracted with 12.5 ml of 10 mM Tris hydrochloride (pH 7.5)-0.05% β-mercaptoethanol-4% dodecyltrimethylammonium bromide (Eastman Kodak Co., Rochester, N.Y.) to remove LPS (6). The sample was centrifuged at 27,000 × g for 40 min, and the sedimented protein was washed once with water and suspended in deionized water and was stored at −20°C.

Analytical methods. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed on 17.5% gels by a previously published procedure (8). Isoelectric focusing experiments were performed in the presence of urea and Nonident P-40 (2). Gels were cast in acid-washed glass tubes (3 mm [inside diameter] by 130 mm [length]) and contained 2% ampholines at pH ranges of 8 to 10, 5 to 7, and 3.5 to 10 in a ratio of 2:2:1 (vol/vol/vol). The anode and cathode buffers were 10 mM H_2PO_4 and 20 mM NaOH, respectively, and electrophoresis was performed at 300 V for 18 h and then at 500 V for 2 h. Gels were stained and destained as
described previously (8) and scanned with a laser densitometer (LKB). Western blotting was performed by the procedure of Towbin and co-workers (31). LPS was detected on SDS-polyacrylamide gels by using a modified silver stain (14).

The total protein concentration was determined by the method of Lowry et al. (21) by using bovine serum albumin as a standard. For amino acid analysis 200 µg of protein was hydrolyzed under vacuum in 6 N hydrochloric acid for 24 h at 110°C. Amino sugars were determined after hydrolysis of the samples in 4 N hydrochloric acid for 5 h at 110°C. Analyses were performed on an analyzer (6300; Beckman Instruments, Inc., Fullerton, Calif.) by the University of Pennsylvania Protein Chemistry Laboratory. Protein hydrophobicity was calculated from the amino acid composition by the methods of Capaldi and Vanderkooi (4) and Hatch (13).

Immunological methods. Immune sera were produced by injecting New Zealand albino rabbits (weight, 3 to 4 kg) subcutaneously in the back with 500 µg of the purified polypeptide emulsified in complete Freund adjuvant (1:1; vol/vol). Booster injections containing the same amount of protein were given 2, 4, and 6 weeks following the first injection. The serum was collected 2 weeks later and was stored at −20°C. Antibody directed against washed, heat-killed cells of *F. nucleatum* ATCC 10953 and FDC 364 was prepared as described previously (8). Double diffusions were performed on agarose slides as described previously (10).

RESULTS

Identification and isolation of the corncob receptor from *F. nucleatum* ATCC 10953. When cell envelope (membrane) and soluble protein fractions of *F. nucleatum* ATCC 10953 were isolated and tested for the ability to inhibit corncob formation, all of the activity resided in the cell envelope (membrane) protein fraction. The membrane fraction was then treated with trypsin and reasayed for corncob-inhibitory activity. The activity of the trypsin-treated membranes was comparable to that obtained with the untreated membrane fraction. SDS-polyacrylamide gel electrophoresis of the untreated membrane, which was solubilized in the presence of SDS at 30°C, revealed a major polypeptide band with an apparent Mr of 41,000 and a number of minor polypeptide bands (Fig. 1, lane A). Only the 41,000-molecular-weight polypeptide displayed heat-modifiable properties when the membrane fraction was solubilized at 50 and 100°C (data not shown). The trypsin-treated sample contained an abundant polypeptide with an apparent Mr of 39,500 (Fig. 1, lane B) which was also heat modifiable (data not shown), suggesting that the polypeptide originated from the 41,000-molecular-weight protein in the untreated membranes.

The receptor activity in the trypsin-treated membrane fraction was extracted with buffered urea and applied to a DEAE-Sepharose column. All of the corncob-inhibitory activity bound to the column and was eluted with 0.5 M sodium chloride. The pooled peak fractions were examined on SDS-polyacrylamide gels and were found to contain the 39,500-molecular-weight polypeptide (Fig. 1, lane C). Results obtained from the analysis of silver-stained gels indicated that the preparation also contained LPS. Amino acid analysis and carbohydrate assays performed on this material showed high concentrations of glucosamine (8 mol/mol of protein) and neutral sugar, respectively. LPS obtained from strain ATCC 10953 was used as a control in these assays. Neither muramic acid nor lanthionine was detected during amino acid analysis and thus confirmed that the protein sample was contaminated with LPS but was free from peptidoglycan. Several unsuccessful attempts were made to remove the LPS by passing the protein sample through anion-exchange (Dowex) and polymyxin B-Sepharose columns. However, extraction of the sample with dodecyltrimethylammonium bromide permitted separation of the detergent-insoluble polypeptide from the solubilized LPS. The final preparation was analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 1, lane D). Scanning densitometry of the gel indicated that the polypeptide represented 14% of the total protein in the cell envelope fraction. The final yield was 13 mg of protein from membranes (536 mg of protein) obtained from 8 liters of culture. The LPS contamination was reduced to 2 mol of glucosamine per mol of protein. The corncob-inhibitory activities of the crude cell envelope fraction (Fig. 2A) and the polypeptide fraction (Fig. 2B) were compared. Inhibition of corncob formation by this polypeptide was dose dependent, with approximately 8 µg of protein exhibiting 50% inhibition. Corncobs formed in the presence of 100 µg of the polypeptide resulted in the binding of 2.6 × 10^5 fewer streptococci than corncobs made in the absence of inhibitor.

To establish that the reagents (trypsin, trypsin inhibitor, urea, and dodecyltrimethylammonium bromide) used in the purification procedure did not cause artificial inhibitory effects in the assay, membrane fractions from *F. nucleatum* FDC 364 and *F. necrophorum* 9432 were carried through the purification procedure, and the resulting protein fractions were examined for corncob-inhibitory activity. Samples

![FIG. 1. Isolation of the corncob receptor polypeptide from *F. nucleatum* ATCC 10953. Samples removed at various steps in the isolation procedure were subjected to SDS-polyacrylamide gel electrophoresis. Lane A, Untreated cell envelope; lane B, trypsin-treated and delipidated cell envelope; lane C, DEAE-bound material eluted with 0.5 M NaCl; lane D, insoluble fraction following extraction with dodecyltrimethylammonium bromide (133 µg of protein). The large arrowhead shows the position of an abundant polypeptide with an apparent Mr of 39,500. All samples were solubilized in the presence of SDS at 50°C. The gel was stained with Coomassie blue. Molecular size standards (in kilodaltons) are designated on the left.](http://iai.asm.org/article-pdf/33/1/329/29089881/329-333)
containing up to 100 µg of protein from each bacterium did not inhibit corncob formation between \( F. \) nucleatum ATCC 10953 and \( S. \) sanguis CC5A (data not shown). In addition, the untreated membrane fraction obtained from strain FDC 364 did not inhibit corncob formation between either this strain or strain ATCC 10953 and \( S. \) sanguis CC5A.

**Properties of the receptor.** Even though extraction of the 39,500-molecular-weight polypeptide with dodecyltrimethylammonium bromide significantly reduced the amount of glucosamine present in the preparation, it had to be established that the sugar that remained did not play a role in receptor activity. Glucosamine, \( N \)-acetylglucosamine, and galactose were tested at various concentrations in the corncob inhibition assay. Reduction of corncob formation was not observed with any of the sugars. Furthermore, LPS extracted from \( F. \) nucleatum ATCC 10953 also failed to inhibit corncob formation.

The amino acid composition of the 39,500-molecular-weight polypeptide is presented in Table 1. The polypeptide had a polarity index of 41%, which is characteristic of hydrophobic membrane proteins, and contained 11% basic residues. The polypeptide had an isoelectric point of approximately 6.6, as determined in urea gels, but displayed some charge heterogeneity on isoelectric focusing and two-dimensional gel analyses, probably because of the presence of bound LPS.

**Prevention of corncob formation with immune serum.** A single diffuse precipitin band was observed in double-diffusion experiments when immune serum was reacted with the isolated 39,500-molecular-weight polypeptide (Fig. 3, well 4). This precipitin band displayed identity with the crude untreated and trypsin-treated cell envelope fractions, as well as with the 0.5 M salt fraction recovered from the}

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**TABLE 1. Amino acid composition of the corncob receptor protein**

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<tr>
<th>Amino acid</th>
<th>No. of residues/polypeptide (nearest integer)*</th>
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<td>Asp</td>
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<tr>
<td>Cys</td>
<td>3</td>
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<tr>
<td>Trp</td>
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*Hydrolysis was for 24 h. The polypeptide (apparent Mr., 39,500) is a trypsin cleavage product.
*ND, Not determined.

DEAE-Sephacel column (Fig. 3, wells 1 to 3, respectively). These results demonstrate that the native major heat-modifiable membrane protein is recognized by antibodies that are made against the trypsin product. Attempts were made to use Western blot analysis to show antigenic cross-reactivity between the native protein and the trypsin product; however, it proved to be difficult to transfer these polypeptides electrophoretically from the acrylamide gel to nitrocellulose or nylon filters. This was apparently because of the hydrophobicity of the polypeptide, as well as LPS binding, since the polypeptide could not be electroeluted from acrylamide gel slices unless SDS was included in the elution buffer. No precipitin bands were noted when the antiserum was reacted with LPS from \( F. \) nucleatum ATCC 10953 (Fig. 3, well 5). In

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**FIG. 3.** Double diffusion experiment showing the precipitin reactions of the various cell fractions prepared during the isolation of the corncob receptor. Well A, Anti-receptor rabbit serum (undiluted); well 1, untreated cell envelope (32 µg); well 2, trypsin-treated and delipidated cell envelope (38 µg); well 3, DEAE-Sephacel fraction eluted with 0.5 M NaCl (60 µg); well 4, dodecyltrimethylammonium bromide insoluble fraction (60 µg); well 5, LPS (35 µg of neutral sugar).
addition, no precipitin reactions were observed when equivalent cell fractions from *F. nucleatum* FDC 364 were reacted with the antiserum. 

The immune serum was examined for its ability to block corncob formation. Immune serum at various dilutions was preincubated with *F. nucleatum* prior to the addition of the streptococci, as in the typical inhibition assay. A dose-dependent inhibition of corncob formation was obtained, with a $10^{-3}$ dilution of antiserum showing close to 50% inhibition (Fig. 4). An antiserum dilution of $5 \times 10^{-2}$ reduced the binding of the streptococci to fusobacteria to a level that was below the autoagglutinated background level. The dilution of antiserum used in this study did not agglutinate suspensions of intact washed cells of *F. nucleatum* ATCC 10953. Furthermore, preimmune rabbit serum and anti-strain FDC 364 serum failed to block the corncob reaction between strain ATCC 10953 and *S. sanguis* CC5A.

Antibodies which recognize various cell surface antigens other than the actual receptor may block cell-cell binding in the coaggregation assay because of steric hindrance. Consequently, this may be interpreted as true inhibition. To determine whether the 39,500-molecular-weight polypeptide could bind to the streptococcal cell surface, a fixed concentration of the polypeptide was preincubated with $2 \times 10^9$ radiolabeled streptococci, and then increasing concentrations of the immune serum were added. The results of this experiment are shown in Fig. 5. As the concentration of antiserum was increased, proportionally more streptococci were agglutinated. The addition of antiserum at a dilution of $1 \times 10^{-2}$ resulted in the agglutination of $3.7 \times 10^8$ more streptococci cells compared with that of polypeptide-coated cells incubated with preimmune serum. Neither the 39,500-molecular-weight polypeptide nor the anti-polypeptide serum alone increased the agglutination of the streptococci above background levels.

**DISCUSSION**

In this study we isolated, but did not purify to homogeneity, a moderately hydrophobic polypeptide that inhibits corncob formation between *F. nucleatum* ATCC 10953 and *S. sanguis* CC5A. The polypeptide was obtained from the cell envelope (membrane) fraction of strain ATCC 10953 by digesting most of the undesired proteins with trypsin. The protein of interest was protected from extensive proteolytic digestion by an apparent tight association with LPS. Kristoffersen and Hofstad (19) have reported that the LPS from strain ATCC 10953 contains glucosamine in the polysaccharide portion of the molecule. The lipid A portion of this LPS also contains N-acetylglucosamine (12), which would be converted to glucosamine following acid hydrolysis. Since no muramic acid or lanthionine (16, 33) was detected during amino acid analysis, the results indicate that the protein sample was contaminated with LPS and not peptidoglycan. Contaminating LPS was reduced by extraction of the tryptic polypeptide with dodecyltrimethylammonium bromide. The polypeptide was recovered as an insoluble pellet. The sensitivity of the fusobacterial-streptococcal coaggregation to detergents (20) precluded their use in extracting the native receptor protein from the cell envelope. Presumably, the dodecyltrimethylammonium bromide does not interfere with corncob formation, because it binds to the LPS rather than to the protein and thus is readily removed during the centrifugation and washing steps. Care was taken to ensure that no binding artifacts were produced during the purification procedure. Since membrane protein fractions prepared from *F. nucleatum* FDC 364, a corncob-forming strain, and *F. necrophorum* 9432 were known not to inhibit coaggregation between strain ATCC 10953 and *S. sanguis*, these strains were subjected to the same extraction protocol as ATCC 10953. The resulting cell fractions did not effect corncob formation, confirming that the extraction buffer components, including urea and dodecyltrimethylammonium bromide, were not carried into the inhibition assay.

Several lines of evidence support the possibility that the isolated polypeptide is one of the specific fusobacterial corncob receptors. The polypeptide inhibited coaggregation of strain ATCC 10953 and *S. sanguis* but failed to inhibit the coaggregation of strain FDC 364 and the streptococci. Antibodies made against the purified polypeptide blocked the
coaggregation of strain ATCC 10953 and S. sanguis but not that of strain FDC 364 or S. sanguis. Antibodies made against heat-killed FDC 364 agglutinated the fusobacteria but did not inhibit corncob formation. Finally, whole streptococcal cells precoated with the polypeptide were agglutinated by the addition of anti-polypeptide serum but not by the addition of preimmune or anti-strain FDC 364 whole-cell serum.

Based on its location in the cell, apparent molecular weight, and heat-modifiable properties, the polypeptide appeared to be the major trypsin product of the F. nucleatum porin protein isolated by Takada and co-workers (29). In that study the anionic detergent lithium dodecyl sulfate was used to solubilize the intact protein. Unfortunately, these investigators did not report an amino acid composition for the porin protein. It was found that, in addition to porin function, the protein exhibited significant B-cell mitogenicity and polyclonal B-cell activation and enhanced the migration of monocytes. We do not know at present whether the polypeptide isolated in our study retains porin activity or displays the immunobiological activities that have been reported for the intact protein. The receptor polypeptide did not appear to be related to the major outer membrane protein (HM-1) that was isolated previously from F. nucleatum FDC 364 (8). The HM-1 protein from strain FDC 364 did not inhibit corncob formation between either strain FDC 364 or ATCC 10953 and S. sanguis. The receptor polypeptide also did not appear to be related to the galactose-binding lectin recently identified in F. nucleatum FN-2 (26). The lectin has an apparent Mₐ of 300,000 to 330,000, as identified in Western blots. In addition, we have shown that up to 150 mM galactose does not inhibit corncob formation between F. nucleatum ATCC 10953 and S. sanguis CC5A.

Interestingly, the physical properties and the amino acid composition of the receptor polypeptide are similar to those of the major outer membrane protein OmpA (3, 4) of Escherichia coli. The OmpA protein has a polarity index of 43 and contains 11% basic residues. It is important that these comparisons are made based on a comparison with the major trypsin product of the native corncob receptor. However, other similarities exist between the two proteins in terms of their physical properties. Both proteins (i) are outer membrane proteins that are exposed on the cell surface, (ii) exhibit anomalous mobilities on SDS-polyacrylamide gels, (iii) appear to bind tightly to LPS, (iv) are relatively resistant to trypsin when bound to LPS, and (v) function as cell surface receptors (for a review of the properties of the OmpA protein see reference 11). Further investigations are required to determine whether there is any homology between the two protein genes.

The in vivo significance of the corncob receptor remains to be determined. It appears that the protein may have evolved multiple functions related to the physiology of the bacterium (porin function) and its ability to colonize the human oral cavity (coaggregation receptor function). Thus, a more detailed characterization of the corncob receptor and the determination of its distribution in oral isolates of F. nucleatum will contribute to our understanding of the role of bacterial coaggregations in plaque formation and gingival inflammation.

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LITERATURE CITED