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Soluble Group- and Type-Specific Antigens from Type III Group B *Streptococcus*

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Two soluble polysaccharide antigens of a type III group B *Streptococcus* were isolated from the culture medium after growth of strain M732 in a chemically defined broth supplemented with acid-hydrolyzed casein. The type- and group-specific antigens were isolated from the culture supernatant by anion-exchange chromatography with diethylaminoethyl-Sephacel. Two carbohydrate-containing peaks, which had serological reactivity with group B or type III antiserum, respectively, were eluted with a linear NaCl gradient and further purified by gel filtration. The type III polysaccharide was found to contain glucose, galactose, glucosamine, and sialic acid, whereas the group B polysaccharide contained galactose, glucosamine, and rhamnose. For the type III polysaccharide, sialic acid was shown to be the major immunodeterminant, and for the group B polysaccharide, rhamnose was the immunodominant sugar. Both the type III and group B polysaccharides were obtained in high yields without employing harsh physical or chemical treatment and both were immunologically distinct. By immunoelectrophoresis or counterimmunoelectrophoresis, type III antigen failed to react with group-specific antiserum and the group B antigen failed to react with type III antiserum.

The group B *Streptococcus* was initially reported as a cause of human disease in 1938 (13), but it was not until the past two decades that these organisms have been recognized as one of the most important causes of neonatal morbidity and mortality (3, 14, 37). Based on acid extracts of cell wall carbohydrates, four serotypes were initially recognized as types Ia, Ib, II, and III (20, 21). Later, strains of the group B *Streptococcus* containing the Ia carbohydrate and the protein antigen, Ibc, were designated as serotype Ic (35). Of the five serotypes associated with neonatal infections, type III is the most frequent isolate (36). Studies indicate that strains of serotype III account for 80% of "early onset" group B disease associated with meningitis and 93% of "late onset" meningitis, which occurs in infants over 10 days of age (4, 11, 23). Because of its clinical importance, the type III group B *Streptococcus* was selected for the studies of antigen isolation and purification described in this report.

The Lancefield procedure of hot HCl extraction of whole cells has been the classic method used to isolate streptococcal antigens. Whereas this method is acceptable for obtaining extracts suitable for typing and grouping strains of streptococci, the product which is obtained has undergone partial degradation. Milder extraction procedures, using cold trichloroacetic acid (23), saline (34), or neutral ethylenediaminetetraacetic acid buffer (5), have been shown to result in larger-molecular-weight antigenic polysaccharides, which retain their acid-labile components, such as sialic acid. For example, Kane and Karakawa (16) isolated a type Ia polysaccharide antigen by boiling the cells in phosphate-buffered saline, pH 7. Treatment of this sialic acid-containing polysaccharide with HCl for 10 min resulted in a polymer devoid of sialic acid, an important immunodeterminant for type Ia and type III polysaccharides (16, 23, 28, 34).

All of the antigens previously described in the literature were derived from intact streptococcal cells. In this study we isolated two soluble polysaccharide antigens of a strain of group B *Streptococcus* directly from the supernatant culture medium. We believe that this experimental approach has the advantage of producing a high yield of undegraded high-molecular-weight antigens suitable for testing as possible vaccines. Use of a completely dialyzable, casein hydrolysate-based medium for growth of the bacteria eliminated the presence of animal proteins that could cross-react with human tissues (32, 38). By using ion-exchange chromatography and gel-filtration, two separate and distinct polysaccharides were isolated and purified. One polysac-

charide carried the determinants of the type III antigen, and the other carried those of the group B antigen.

MATERIALS AND METHODS

Bacterial strains. Reference strains of group B streptococci type Ia (090/14), type Ib (H36B/60/2), type Ibc (A909/14), type II (18RS21/61/1), and type III (D136C) were obtained from Rebecca Lancefield, The Rockefeller University, New York, N.Y. Strain 090R, which lacks type-specific antigen, was obtained from Richard Facklam, Center for Disease Control (CDC), Atlanta, Ga. Strain M732, isolated from an infant with meningitis, was obtained from Dennis Kasper, Harvard Medical School, Boston, Mass.

All strains were received in a lyophilized state and were rehydrated in Todd-Hewitt broth (THB; BBL Microbiology Systems, Cockeysville, Md.) and incubated overnight at 37°C. The stock strains were stored frozen at −70°C in THB with 20% glycerol and in the lyophilized state in THB.

Growth medium. All experiments were conducted in a modification of the chemically defined medium of Terleckyj et al. (30). In this medium (Table 1), acid-hydrolyzed casein was substituted for many of the individual amino acids and the concentration of sodium phosphates was increased to 0.16 M.

TABLE 1. *Chemical composition of the casein hydrolysate-based medium*

Compound	Concn per liter
Acid-hydrolyzed casein (Humko Sheffield, Norwich, N.Y.)	5.0 g
Dextrose (Pfansteihl Labs, Waukegan, Ill.)	40.0 g
KH_2PO_4	0.44 g
K_2HPO_4	0.31 g
Ammonium sulfate	0.6 g
$NaH_2PO_4 \cdot H_2O$	8.2 g
Na_2HPO_4	12.6 g
Sodium acetate, anhydrous	6.0 g
Sodium citrate	0.225 g
L-Glutamine	0.3 g
L-Asparagine	0.3 g
L-Tryptophan	0.1 g
L-Cystine	0.2 g
L-Cysteine HCl·H_2O	0.65 g
Riboflavin	0.8 g
DL-Panthotenic acid	1.72 mg
Thiamine HCl	0.8 mg
Para-aminobenzoic acid	0.16 mg
Nicotinic acid amide (nicotinamide)	4.0 mg
Biotin	0.02 mg
Pyridoxamine·2HCl	2.3 mg
Folic acid	0.2 mg
Adenine sulfate	21.75 mg
Guanine HCl	15.5 mg
Uracil	12.5 mg
$MgSO_4 \cdot 7H_2O$	0.4 g
NaCl	20.0 mg
$FeSO_4 \cdot 7H_2O$	20.0 mg
$MnSO_4 \cdot H_2O$	15.1 mg

The medium was adjusted to pH 7.0 with NaOH and sterilized by filtration through a 0.45-μm membrane filter. Before inoculation, a freshly prepared solution of 8.4% sodium bicarbonate was filter sterilized and added to the medium at a concentration of 10 mg/liter. All chemicals were obtained from either J. T. Baker Chemical Co., Phillipsburg, N.J., or GIBCO Laboratories, Grand Island, N.Y., unless otherwise indicated.

Growth conditions. From a frozen culture, strain M732 was inoculated onto a Trypticase soy blood agar plate which was incubated overnight at 37°C. From this plate, 10 colonies were selected to inoculate 100 ml of the casein-based broth. After 6 h at 37°C, the starter culture was transferred into 20 liters of the casein-based broth, which was incubated at 37°C for 48 h without stirring and without adjusting the pH of the medium. Purity of all cultures was ascertained by streaking the culture onto Trypticase soy blood agar plates after each transfer and before harvesting the final culture.

Isolation and purification of group and type antigens. The cells were separated from the culture supernatant by filtration with a 0.45-μm filter (HAWPOHV20), using the Pellicon cassette system (Millipore Corp., Bedford, Mass.). The culture supernatant was concentrated 40-fold and dialyzed by using the Pellicon cassette equipped with a 10,000 nominal-molecular-weight-limit filter cassette (PTG00005). All procedures were carried out at 4°C, after which the retentate was heated at 60°C for 30 min to inactivate any enzymes present which might alter the antigens.

The initial purification of group and type antigens was by ion-exchange chromatography. The retentate was mixed with 100 ml of diethylaminoethyl-Sephacel (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) in 0.01 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.0, and allowed to stand overnight at 4°C. The mixture was poured into a column (2.4 by 20 cm), and the antigens were eluted by a linear gradient of 0 to 0.75 M NaCl in a total volume of 1,200 ml of 0.01 M Tris buffer, pH 7.0.

Fractions containing material reactive with type-specific antiserum were pooled, concentrated in an ultrafiltration cell (Amicon Corp., Lexington, Mass.), and dialyzed. Approximately 4 ml of the concentrate was placed on a Sepharose 4B column (Pharmacia) (2.5 by 80 cm) with a volume of 400 ml. The column was calibrated with blue dextran 2000 and dextran T500 (M_w = 511,000) (Pharmacia). The antigen was eluted with either 0.2 M ammonium acetate buffer with 0.02% sodium azide (pH 7.0) or 0.01 M Tris buffer with 0.02% sodium azide (pH 7.3). Fractions containing material reactive with group-specific antiserum were processed in the same manner. All column fractions were analyzed for their total carbohydrate content by the phenol-sulfuric acid assay (10), for their protein content by the Lowry procedure (25), and for their serological reactivity by capillary precipitation by the method of Lancefield (22).

Fractions containing either group or type antigens were pooled separately and lyophilized to measure the yield of each antigen. All antigenic material was stored either in the lyophilized state or kept frozen at −70°C.

Hot HCl extraction of type III antigen. Cells of

strain M732, grown in THB, were treated with 0.2 N HCl at 100°C for 10 min by the method of Lancefield (22). After neutralization, the cells were removed. Four volumes of alcohol were added to the antigen-containing supernatant at 4°C to precipitate both the group- and type-specific antigens. The precipitate was dissolved in saline, and the type III antigen was precipitated with one volume of alcohol.

Isolation and purification of type III native polysaccharide. The "native" polysaccharide antigen from strain M732 was prepared by the method described by Baker et al. (5).

Preparation of antisera. Group- and type-specific antisera were raised in 5-lb (ca. 2.268-kg) New Zealand white rabbits by the procedure of Lancefield (22, 24). The whole-cell vaccine to elicit group-specific antibodies was a 3% formalinized culture of strain 090R; the type III-specific vaccine was a 0.3% formalinized culture of strain D136C. After immunization, the rabbits were bled by cardiac puncture and all sera were stored at −70°C.

Reference antisera to the five serotypes of group B streptococci were generously provided by Rebecca Lancefield. Group-specific rabbit antiserum to the group B *Streptococcus* was obtained from the CDC.

Serological methods. The antigen preparations were characterized according to their immunological reactions by capillary precipitation (22) and their diffusion in agar-gel by the method of Ouchterlony (27). The electrophoretic mobility was determined by the method of Scheidegger (29), using the Hyland electrophoresis kit (Hyland Laboratories, Inc., Costa Mesa, Calif.). The immunoelectrophoresis was run at 30 mA for 40 min at 4°C in barbital buffer, pH 8.6.

Counterimmunoelectrophoresis was performed at 6 V/cm for 35 min in barbital buffer, pH 8.6. A clean glass slide (5 by 5 cm) was covered with 3.5 ml of 1% agarose. Holes were punched in the gel approximately 4 mm in diameter and 5 mm apart, and 9 μl of either antigen or antiserum was added. At the end of the run, the gels were examined for bands of precipitate.

Quantitative precipitation inhibition tests. Inhibition of quantitative precipitins was performed by the procedure described by Knox et al. (19), using 10 μg of the type III or 5 μg of the group B polysaccharide. A 100-μl amount of type III-specific antiserum or 50 μl of group B antiserum was tested with 0, 1, 5, 20, and 40 μmol of D-glucose, D-galactose, L-rhamnose, *N*-acetylglucosamine, and *N*-acetylneuraminic acid. The total antibody protein precipitated was determined by the method of Lowry et al. (25).

Biochemical characterization of the antigens. The qualitative sugar composition was determined by descending paper chromatography with 0.2 to 1 mg of each polysaccharide, which had been hydrolyzed in 4 N HCl for 4 h at 100°C. The material was dried, rehydrated in water, reduced again to dryness, and dissolved in 25 μl of water and spotted onto Whatman no. 1 filter paper. Standards of glucose, galactose, rhamnose, ribose, mannose, glycerol, glucosamine, galactosamine, and glucuronic acid were made as 2% solutions in 20% ethanol and spotted on the same paper as the polysaccharide antigens. The acid hydrolysates were resolved with a solvent system of *n*-butanol-pyridine-water (6:4:3) run at room temperature for 17 h. The papers were dried and stained with silver nitrate-sodium hydroxide (31).

The partial chemical composition of the type- and group-specific antigens was determined by the phenol-sulfuric acid assay for neutral sugars (10), the Lowry protein assay with bovine serum albumin as the standard (25), and the thiobarbituric acid assay for sialic acid content (2, 33). Antigens were also analyzed for the presence of rhamnose (9), phosphorus (1), and nucleic acids (6, 15).

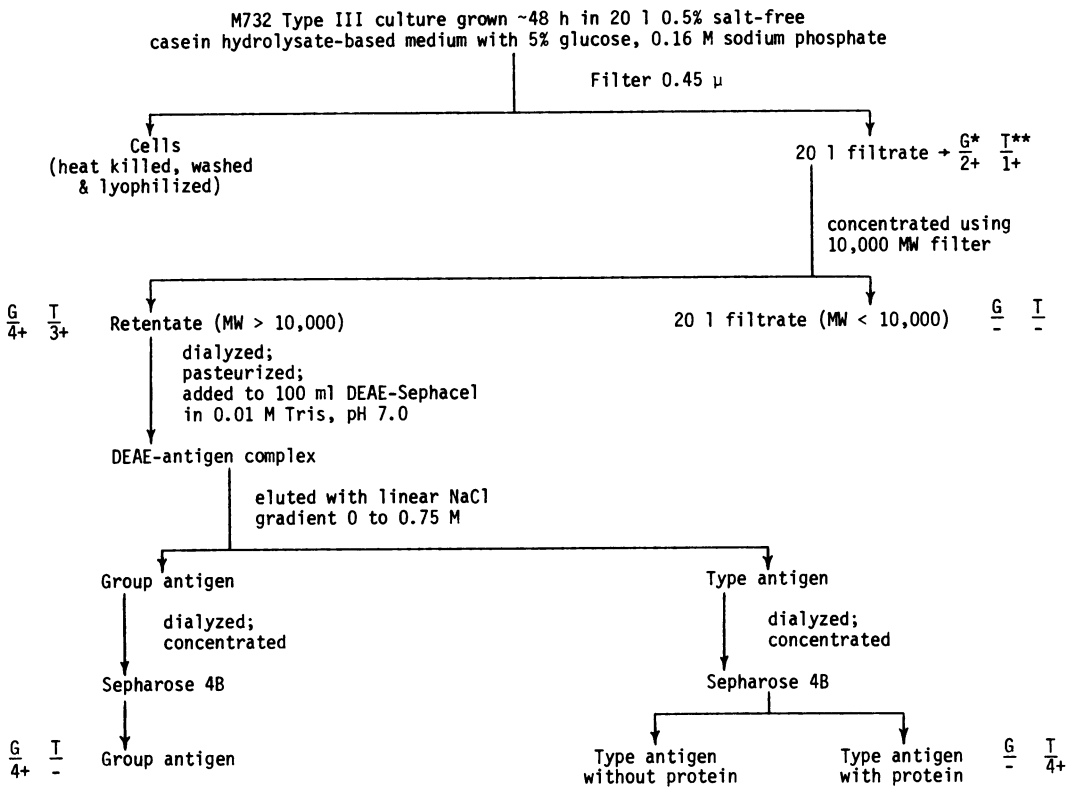
RESULTS

Isolation of the soluble antigens from the culture supernatant. The procedure by which two separate and distinct polysaccharide antigens of the type III group B *Streptococcus* were isolated from the culture medium after growth of strain M732 is outlined in Fig. 1. Before concentration, the presence of type III and group B determinants in the cell-free supernatant was detected by the capillary precipitin test. Unconcentrated culture medium gave a 1+ reaction with type III antiserum. After a 40-fold concentration and dialysis, the type III serological reactivity increased to a 3+ reaction. Reaction of group B antiserum with the concentrated supernatant medium gave little precipitate due to antigen excess. Maximal precipitation was obtained when the supernatant was diluted 1:32.

The antigenic polysaccharides, retained by the filter (molecular weight > 10,000), were bound to diethylaminoethyl (DEAE)-Sephacel. The resin was poured into a column, which was eluted with a linear NaCl gradient (Fig. 2). Two carbohydrate peaks were detected. The first peak, which eluted between 0.02 and 0.15 M NaCl, contained group B serological reactivity, and the second peak, which contained type III reactivity and little group B reactivity, eluted between 0.15 and 0.22 M NaCl. A low level of protein was detected in all of the polysaccharide-containing fractions. Fractions 16 to 35 (group B reactive) and fractions 45 to 55 (type III reactive) were separately pooled, and each was purified on a Sepharose 4B column (Fig. 3 and 4).

The polysaccharide containing the type III determinant eluted from the Sepharose 4B column as a single, nearly symmetrical, carbohydrate-containing peak (Fig. 3). Because of the slightly higher protein content in the later fractions, fractions 46 to 69 (194 to 287 ml), which contained negligible protein (2.1% of dry weight), and fractions 70 to 79 (297 to 340 ml), which contained some detectable protein (7.4% of dry weight), were pooled separately. The total yield of type III polysaccharide from a 20-liter culture was 34.5 mg, dry weight.

Carbohydrate material with group B serological reactivity was eluted from the Sepharose 4B



* G = Group antigen concentration expressed as capillary precipitin titer.
** T = Type antigen concentration expressed as capillary precipitin titer.

FIG. 1. *Schematic diagram of the procedure used for the isolation and purification of type III and group B polysaccharide antigens from strain M732.*

column as two overlapping peaks (Fig. 4). The first carbohydrate-containing peak contained nearly all of the group B serological reactivity and no detectable type III antigen. The small, secondary peak of group B reactive material was pooled separately because of its smaller molecular size. From the major peak, the yield of purified group B polysaccharide was 87 mg, dry weight.

The apparent molecular size of the type and the group polysaccharides was estimated by gel filtration on a Sepharose 4B column eluted with 0.01 M Tris buffer (pH 7.3) to compare our data with those reported in the literature (5). Dextran T500 was used to determine the molecular weight distribution characteristics of the column. The type III polysaccharide was eluted at a K_{av} (partition coefficient) of 0.30, which is consistent with a molecular weight of 525,000. For the group B polysaccharide, a K_{av} of 0.63 was extrapolated to indicate a molecular size of 115,000.

Chemical composition of the soluble polysaccharide. Paper chromatography was used to detect the presence of galactose, glucosamine, and rhamnose in the group B polysaccharide. The Dische and Shettles reaction (9) indicated that rhamnose composed 62% of the group B polysaccharide. The group B polysaccharide also contained 2% phosphorus and 1.2% protein.

Glucose, galactose, glucosamine, and sialic acid were detected in the type III polysaccharide. The sialic acid content of the type III polysaccharide was found to be 26% by the thiobarbituric acid assay (33). The presence of deoxyribonucleic acid and ribonucleic acid was not detected by the diphenylamine (6) and the orcinol (15) reactions, respectively, in either of the purified antigen preparations.

Immunological properties of the soluble polysaccharides. Type III and group B antisera prepared in several different rabbits reacted by capillary precipitation and agar-gel diffusion with their homologous antigens. Purified type

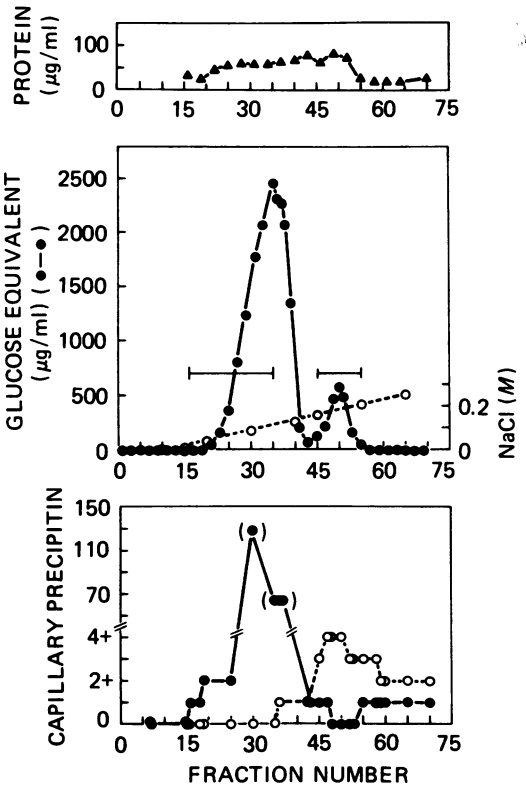


FIG. 2. *Separation of type III and group B polysaccharides on a DEAE-Sephacel column with a linear NaCl gradient in 0.01 M Tris buffer, pH 7.0. Capillary precipitins were performed with type III antiserum, O--O, and group B antiserum, ●——●. (●) represents the dilution of the material × 4+ group B precipitation. Bars indicate the fractions which were pooled and concentrated for further purification.*

III polysaccharide precipitated only with type III antiserum, and the group B polysaccharide reacted only with group B antiserum.

Migration toward the anode after immunoelectrophoresis showed that both purified polysaccharides had a negative charge. The type III polysaccharide consistently migrated more rapidly than the group-specific polysaccharide, and each polysaccharide formed a precipitin arc only with its homologous antiserum (Fig. 5). The material extracted from the cells of strain M732 by the hot HCl method of Lancefield (22), when reacted against type III antiserum, showed precipitin bands which migrated slightly toward the cathode, suggesting that it may have a slight positive charge. When the same material was reacted against group B antiserum, a line of precipitate was observed that had moved toward the anode. The native type III polysaccharide that we prepared by the neutral-buffer extraction procedure of Baker (5) contained material that migrated similarly to the type III polysaccharide that we isolated and purified from the culture supernatants. However, the native type III polysaccharide formed a precipitin arc, not only with the type III antiserum, but also with the group B antiserum, which appeared as a band which had migrated toward the anode.

Counterimmunoelectrophoresis confirmed the serological specificity of the two polysaccharide antigens that we isolated from the culture medium. Our type III polysaccharide preparation reacted with the type III-specific antiserum as a discrete line of precipitate, but failed to show a precipitin line with the group B antiserum (Fig. 6). The purified polysaccharide-containing group B determinants failed to react with the type III antiserum but reacted with its homologous antiserum. As expected from the results of the immunoelectrophoresis described previously, type III antigens prepared by the method of Lancefield (22) or Baker (5) gave a precipitin band with both the type- and group-specific antisera.

Immunodeterminants of the soluble polysaccharides. Quantitative precipitin reactions between 100 μl of type III antiserum and 10 μg

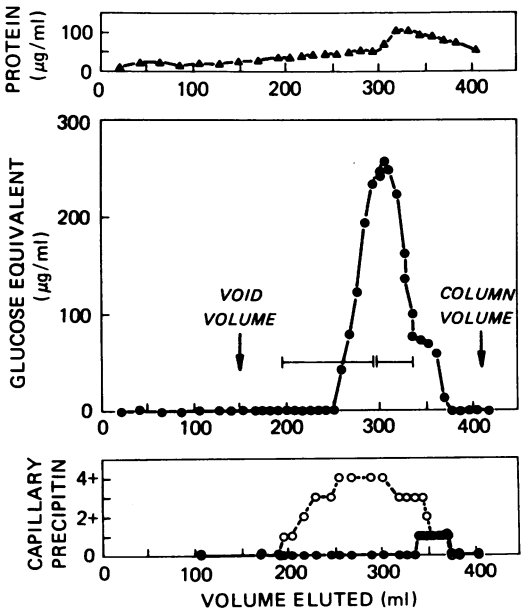


FIG. 3. *Gel filtration of type III polysaccharide on a Sepharose 4B column in 0.2 M ammonium acetate buffer, pH 7.0. Capillary precipitins were performed with type III antiserum (O--O) and group B antiserum, ●——●. Bars indicate the fractions which were pooled.*

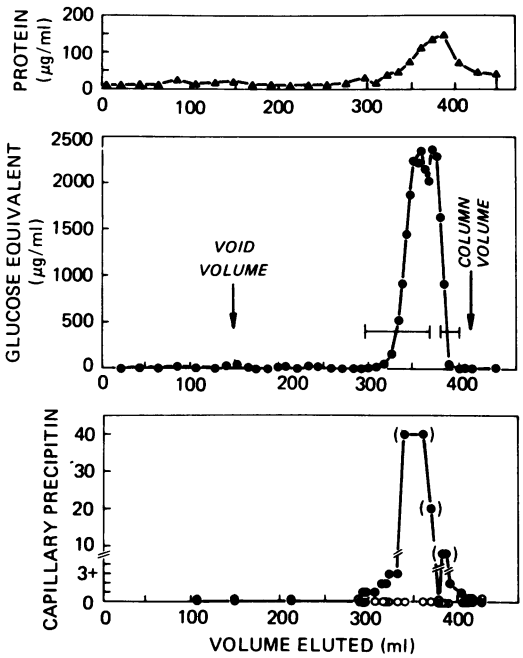


FIG. 4. *Gel filtration of group B polysaccharide on a Sepharose 4B column in 0.2 M ammonium acetate buffer, pH 7.0. Capillary precipitins were performed with type III antiserum, O- - -O, and group B antiserum, ●——●. (●), Dilution of the material × 4+ group B precipitation. Bars indicate the fractions which were pooled.*

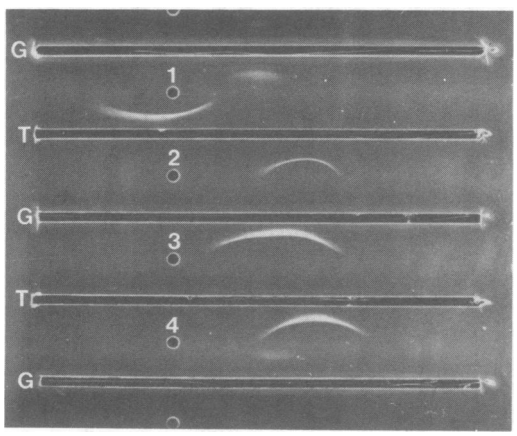


FIG. 5. *Immunoelectrophoresis of the purified polysaccharide antigens of strain M732. Well 1, purified hot HCl-extracted type III antigen; well 2, purified type III antigen from the culture supernatant; well 3, purified group B antigen from the culture supernatant; well 4, purified EDTA-extracted native type III antigen. Troughs contain either group B antiserum (G) or type III antiserum (T), prepared in rabbits to formalinized whole-cell vaccines.*

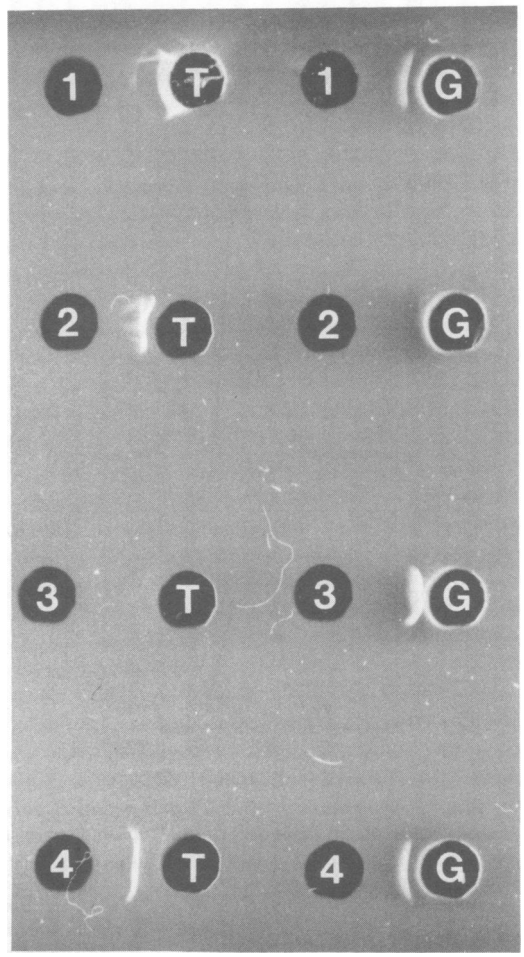


FIG. 6. *Counterimmunoelectrophoresis of the purified polysaccharide antigens of strain M732 against type III antiserum (T) or group B antiserum (G). Row 1 shows EDTA-extracted native type III antigen; row 2 shows purified type III antigen from the culture supernatant; row 3 shows purified group B antigen from the culture supernatant; row 4 shows purified hot HCl-extracted type III antigen.*

of purified type III polysaccharide were inhibited 50% by 4 μmol of *N*-acetylneuraminic acid (Fig. 7B) and were maximally inhibited (95%) by 20 μmol of *N*-acetylneuraminic acid. These results are consistent with other data (17) that this amino sugar is the major determinant of the type III antigen. D-Glucose, D-galactose, L-rhamnose, and *N*-acetylglucosamine, at concentrations up to 40 μmol, all failed to inhibit this precipitation reaction.

Similarly, quantitative precipitation reactions between 50 μl of group B antiserum and 5 μg of purified group B polysaccharide were inhibited

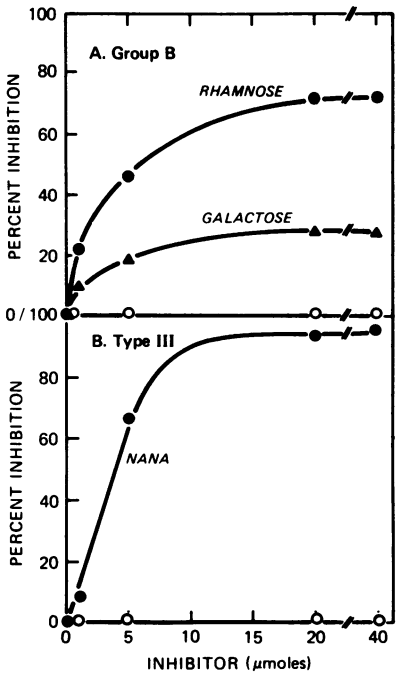


FIG. 7. *(A) Inhibition of precipitation between 5 μg of group B polysaccharide and 50 μl of homologous antiserum. Inhibitors were ●, L-rhamnose; ▲, D-galactose; ○, D-glucose, N-acetylglucosamine, or N-acetylneuraminic acid. (B) Inhibition of precipitation between 10 μg of type III polysaccharide and 100 μl of homologous antiserum. Inhibitors were ●, N-acetylneuraminic acid (NANA); ○, L-rhamnose, D-glucose, D-galactose, or N-acetylglucosamine.*

50% by 6 μmol of L-rhamnose and were maximally inhibited (72%) by 20 μmol of L-rhamnose (Fig. 7A). These findings are in accord with previous data (8) that rhamnose is the major determinant of the group-specific polysaccharide. D-Galactose at 40 μmol also inhibited the group B precipitin reaction by 27%, whereas D-glucose, *N*-acetylneuraminic acid, and *N*-acetylglucosamine failed to inhibit the reaction at concentrations up to 40 μmol.

DISCUSSION

Two soluble polysaccharides, one, a rhamnose-containing polysaccharide possessing the determinants of the group B streptococcal antigen, and the other, a neuraminic acid-containing polysaccharide possessing the determinants of the type III antigen, were isolated and purified from the supernatant medium after growth of strain M732. This is the same strain of type III group B *Streptococcus* used by Baker et al. (5) to obtain native type III polysaccharide antigen by extraction of the cells with a neutral ethylenediaminetetraacetic acid (EDTA) buffer. Whereas Baker et al. grew the cells in a modified THB, we used a medium containing acid-hydrolyzed casein and chemically defined components to eliminate from the final product non-dialyzable components and substances of animal origin that might possibly cross-react immunologically with human tissues. The methods used to isolate these polysaccharides from the culture medium did not involve any chemical treatments, so that the resultant products should be extremely close to those produced by the organism during colonization and infection.

The mechanism by which these two polysaccharides appear in the culture medium is not known. The presence of extracellular, non-peptidoglycan polysaccharides has been observed with other streptococci, such as the presence of the serotype *a* antigen in the medium after growth of *Streptococcus mutans* AHT (7). It is unlikely that the large amounts of extracellular polysaccharide found are the result of cellular lysis, since lysis of a substantial fraction of the cells would be required and decreases of cellular turbidity after the exponential growth phase were not observed. Furthermore, evidence for the presence of increasing levels of both group B and type III polysaccharides during exponential growth has been obtained (S. Dillon et al., manuscript in preparation). Two other hypotheses can be considered. It is possible that the polysaccharide covalently linked to wall peptidoglycan is lost via selective hydrolysis of a few bonds or that these extracellular polysaccharides could represent an excess of assembled polysaccharide chains that fail to become covalently linked to the cell wall peptidoglycan during the assembly process.

The type III polysaccharide was eluted from the Sepharose 4B column in 0.01 M Tris buffer at a K_{av} of 0.30, suggesting an apparent molecular size of 525,000. This is similar to the ethylenediaminetetraacetic acid non-pH-titrated type III antigen of Kasper et al. (18), reported to have a molecular size of 650,000 (K_{av} = 0.28). When their cells were grown in a pH-titrated medium, the EDTA-extracted polysaccharide appeared to be much larger. In contrast, when we grew the same strain of group B *Streptococcus* (M732) in our medium at a constant pH of 7.0, no increase in molecular size was seen; on the contrary, the type III polysaccharide eluted at a K_{av} less than 0.30, indicating a molecular size smaller than 525,000. This smaller molecular size may have been the result of enzymatic degradation of the polysaccharide antigen. Group B streptococcal enzymes, such as neuraminidase, have been found in the culture medium (26) and may

be enzymatically active at a neutral pH.

Kasper et al. (18) gave no estimate for the molecular size of the group-specific polysaccharide. The group B polysaccharide isolated in our study had a K_{av} of 0.63. It is important to note that the molecular weights of these polysaccharides determined by gel filtration are only relative approximations since they are not globular molecules. The discrepancy of elution volumes of the polysaccharides with two different buffers is indicative of the problem. When the column was eluted with 0.2 M ammonium acetate, the K_{av} of the type III polysaccharide was 0.51 and the K_{av} of the group B polysaccharide was 0.85.

The results presented in this paper show that both the type III polysaccharide and the group B polysaccharide were obtained in a highly purified state in reasonably high yield. From a 20-liter culture in casein hydrolysate-based medium, 87 mg of group B polysaccharide and 34.5 mg of type III polysaccharide were obtained. The yield of our type III polysaccharide was 30-fold higher than the yield of native type III antigen originally reported by Baker et al. (5). In later reports, their yield of native type III polysaccharide was increased to 20 to 60 mg by growing cells in 20 liters of pH-titrated-modified THB (18).

Quantitative precipitation of the group B polysaccharide with group-specific antiserum was inhibited by low concentrations of L-rhamnose. By the Dische and Shettles assay (10), approximately 62% of the group B polysaccharide was rhamnose. These data are consistent with the previous work of Curtis and Krause (7) which showed that the formamide-extracted group B antigen contained 50% rhamnose, which was found to be the immunodominant sugar. These results were further substantiated by Freimer's study of the type II group B *Streptococcus* (12). The partial inhibition (27%) of precipitation was observed between the group B polysaccharide and the homologous antiserum with 40 μmol of D-galactose. Curtis and Krause (8) also observed partial inhibition of the group B precipitation reaction by D-galactose (26.3%) and several other monosaccharides. They suggested that a sugar was an effective inhibitor if it possessed the steric arrangement at carbons 2 and 4 similar to L-rhamnose.

The methods described in this paper to isolate and purify the type- and group-specific polysaccharide antigens of strain M732 are applicable to other strains of the group B *Streptococcus*. Doran et al. (submitted for publication) have grown a type III group B *Streptococcus*, strain 110, in a chemically defined medium. The supernatant fluid was found to contain two separate peaks of type III polysaccharide material. One peak contained a high-molecular-weight polysaccharide which reacted with type-specific antiserum, and the other peak contained low-molecular-weight material which lacked serological reactivity. The elution of a peak of low-molecular-weight polysaccharide was not observed in our DEAE ion-exchange chromatography. Although these investigators reported the presence of group B polysaccharide in large quantities in their supernatant medium, the polysaccharide was not further characterized. Experiments in progress in our laboratory with clinical isolates and stock strains of the type II group B *Streptococcus* indicate that the procedure described in this report is suitable for the isolation and purification of the polysaccharide antigens of the other serotypes of the group B *Streptococcus*.

In summary, the procedures described in this report permit recovery of a high yield of purified group B and purified type III polysaccharides without harsh chemical or physical manipulation. Thus, these polysaccharide antigens should be immunochemically closer to the antigens present in the body fluids during naturally occurring infections with the group B *Streptococcus* than those antigens chemically extracted from cells. The two polysaccharides were distinct molecular entities which did not cross-react immunologically. It is anticipated that polysaccharides derived from the culture medium can be used as candidate vaccines and as reagents in epidemiological studies to evaluate levels of antibody to group B streptococci.

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