INFECTION AND IMMUNITY, Apr. 1978, p. 194-199
0019-9567/78/0020-0194/$02.00/0

Vol. 20, No. 1
Printed in U.S.A.

Filamentous Capsulated Streptococci from the Human Respiratory Tract: Chemical and Immunochemical Characterization of the Polysaccharide Capsular Antigen of Provisional Binary Capsular Type 87

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Received for publication 19 December 1977

The polysaccharide capsular antigen of the filamentous binary capsulated streptococcus of provisional type 87 and the polysaccharide capsular antigens of two pneumococcal strains transformed with deoxyribonucleic acid of streptococcus type 87 have been purified and analyzed with regard to their component monosaccharides. The purified polysaccharides from the three strains were immunochemically identical. Each was found to contain rhamnose, glucose, galactose, galactosamine, and phosphate. Rhamnose was the immunodominant sugar.

In the previous report (5), the isolation and some of the properties of the filamentous streptococcus of provisional type 87, isolated from the human respiratory tract, were described. Investigation of this organism and of pneumococcal transformants, obtained by exposure of noncapsulated variants of the latter species to crude preparations of deoxyribonucleic acid from the former, revealed streptococcus type 87 to be a binary capsulated organism, one of the capsular components of which is a glycoprotein. In the present report, the isolation, purification, and analysis of the other capsular component of streptococcus type 87, a polysaccharide, is described.

MATERIALS AND METHODS

Bacterial strains, media, and preparation of antisera. The sources and methods are described in the accompanying report (5).

Isolation of polysaccharide capsular antigens. Streptococcus type 87 and transformed pneumococcal strains A66R2T87S and R36NCT87S were grown to late exponential phase in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) (1). The cells of streptococcus type 87, harvested after centrifugation, were extracted with 10% trichloroacetic acid at 4°C for 4 h, a procedure having been shown previously to destroy the glycoprotein capsular antigen of this organism (5). Cells of transformed pneumococcal strains A66R2T87S and R36NCT87S were autolyzed as described in the previous report (5) before extraction with 10% trichloroacetic acid in order to increase the yield of capsular antigen. Trichloroacetic acid extracts of each organism were dialyzed against three changes of 100 volumes of distilled water after removal of cellular detritus by centrifugation and were lyophilized. Further purification of the antigens was achieved by chromatography on diethylaminoethyl (DEAE)-cellulose and by elution with a linear gradient of 0.02 to 0.5 M $(NH_4)_2CO_3$. In some instances, the material was subjected to gel filtration on a Sephadex G-200 column.

Analytical and serological methods. The techniques used were those described previously (5). Immunoelectrophoresis was carried out according to the method described by Rosan (4).

Infrared spectroscopy. Samples of purified polysaccharide were ground together with KBr and pressed into a plate in a die for a scan of the infrared spectrum in a Beckman IR-18X spectrophotometer.

RESULTS

Isolation and purification of the polysaccharide capsules of streptococcus type 87 and of its pneumococcal transformants. Chromatography on DEAE-cellulose of the extract obtained from cells of streptococcus type 87 with 10% trichloroacetic acid gave rise to three peaks (Fig. 1), of which only the major one precipitated with antiserum to streptococcus type 87 and to pneumococcus A66R2T87S in an agar gel (Fig. 2), giving lines of identity with each antiserum. This reactive fraction was used subsequently in the chemical and immunochemical analyses of the capsular antigen. Capsular polysaccharide was extracted also from intact cells and from lysates of the transformed pneumococcal strains A66R2T87S and R36NCT87S. A high yield of antigen was obtained after treatment of lysates with 10% trichloroacetic acid at 4°C for 1 h, and the preparations to be described were obtained in this fashion. Acid extracts of each strain were chromatographed on DEAE-cellulose. The extract of strain A66R2T87S gave

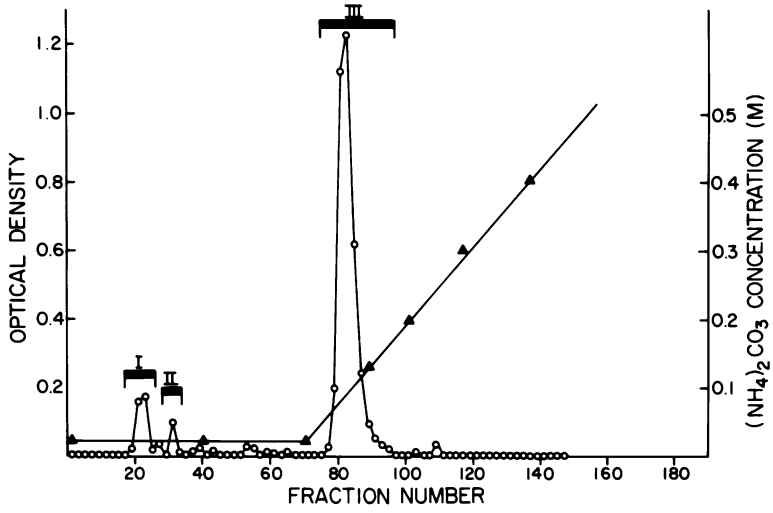

FIG. 1. *Purification of the polysaccharide capsular antigen of streptococcus type 87 by chromatography on a DEAE-cellulose column. $(NH_4)_2CO_3$ gradient (▲); total carbohydrate values by phenol-sulfuric acid assay (optical density at 490 nm) (○). Roman numerals indicate pooled fractions.*

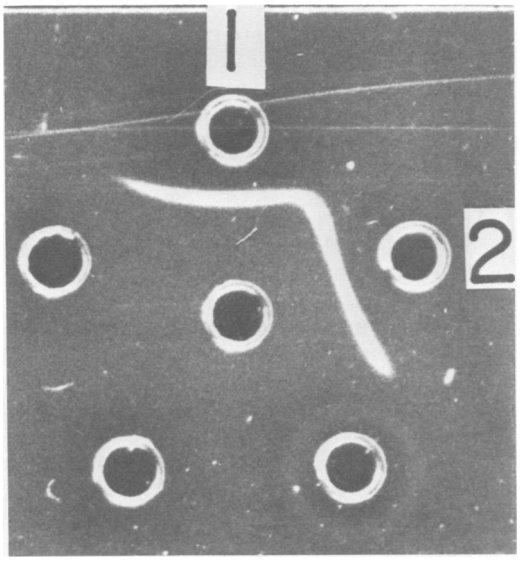

FIG. 2. *Precipitin reactions of pool III of the polysaccharide capsular antigen from streptococcus type 87 (center well) with antisera to streptococcus type 87 and to pneumococcus A66R2T87S in outer wells 1 and 2, respectively.*

two peaks poorly separated from one another (Fig. 3), each of which was divided into two pools. All four pools gave lines of precipitate in agar gels with antisera to streptococcus type 87 and to transformed pneumococcus A66R2T87S. Pool IV, which had the lowest content of phosphate, was refractionated on Sephadex G-200 (Fig. 4). Again four pools, IVa, IVb, IVc, and IVd, were isolated and tested for precipitation in agar gels with the antisera just cited as well as with antiserum to the noncapsulated parental pneumococcal strain A66R2 (Fig. 5). Because pool IVa showed negligible reactivity with the latter antiserum, indicating a minimal content of pneumococcal constituents of other than capsular origin, it was used for comparative studies with the capsular polysaccharide of streptococcus type 87. The antigen isolated from the trichloroacetic acid extract of transformed pneumococcal strain R36NCT87S yielded only a single peak of polysaccharide after chromatography on DEAE-cellulose. Material from this peak gave precipitin lines of identity with the polysaccharide capsular antigen of streptococcus type 87 and the capsular antigen of pneumococcus A66R2T87S with antiserum to either organism (Fig. 6) in an Ouchterlony agar gel.

Immunoelectrophoretic analysis of the purified polysaccharide antigens from streptococcus type 87 and transformed pneumococcus A66R2T87S with antisera to the two organisms (Fig. 7) revealed identical electrophoretic mobility of the two antigens.

Chemical analysis of the polysaccharide antigens. Quantitative analysis of the monosaccharides comprising the capsular polysaccharides of streptococcus type 87 and of the two pneumococcal transformants was performed by gas chromatography after hydrolysis. The monosaccharides were converted to their alditol acetates and, in the case of amino sugars, to their anhydroalditol acetates after deamination. Amino acids were assayed in an automated amino acid analyzer (Table 1). The purified capsular polysaccharide from each of the three

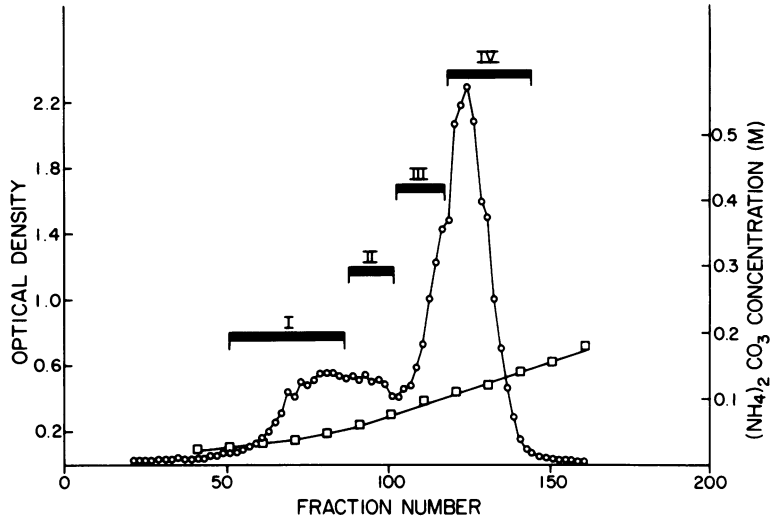

FIG. 3. *Purification of the polysaccharide capsular antigen from pneumococcus A66R2T87S by chromatography on a DEAE-cellulose column. $(NH_4)_2CO_3$ gradient (□); total carbohydrate by phenol-sulfuric acid assay (optical density at 490 nm)* (○). *Roman numerals indicate pooled fractions.*

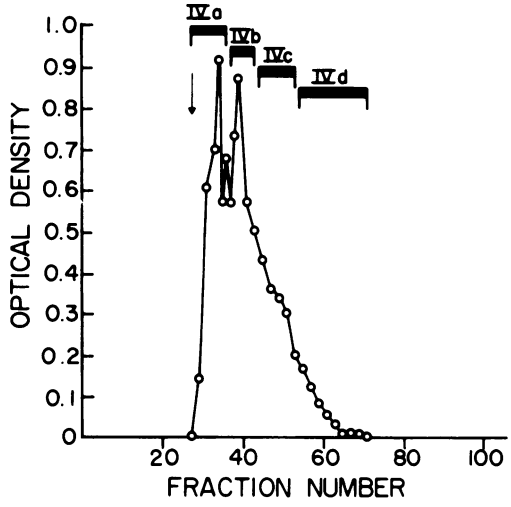

FIG. 4. *Gel filtration on Sephadex G-200 of pool IV of the extract of the polysaccharide capsular antigen of pneumococcus A66R2T87S from the DEAE-cellulose column (Fig. 3). Total carbohydrate values by phenol-sulfuric acid assay (optical density at 490 nm)* (○); *void volume* (↓). *Pooled fractions are designated by IVa, IVb, IVc, and IVd.*

strains contained rhamnose, glucose, galactose, galactosamine, and phosphate. All contained traces of amino acids, which remained associated with the carbohydrate moiety even after extensive purification and digestion with proteases. A similar association has been described in the carbohydrate antigen of *Streptococcus mutans* by Iacono et al. (2).

To determine the terminal and potentially immunodominant monosaccharides of the polysaccharide capsular antigen, timed hydrolysis of the purified fractions of streptococcus type 87 and of pneumococcus A66R2T87S was carried in 0.1 N HCl at 100°C for periods of 10 to 60 min, followed by paper chromatography of the hydrolysates (3). Examination of the chromatograms revealed rhamnose to be the first monosaccharide released, followed by galactose, glucose, and galactosamine.

Infrared spectroscopy of the polysaccharide antigens. To determine whether or not the capsular antigen contains any *O*-acyl groups (ester linkages), infrared spectra of the purified fractions of the polysaccharide antigens of streptococcus type 87 and of pneumococcus A66R2T87S were obtained (Fig. 8). The absence

of O—C—R groups is indicated by the absence of an absorption band between 1,750 and 1,700 cm^{-1}. The presence of an absorption band at 1,690 cm^{-1}, however, suggests the presence of

secondary amide (—NH—C—) linkages which could result from the presence of either *N*-acetylgalactosamine or peptide links of the amino acids present or both.

Serological determination of the immunodominant determinant of the capsular antigen. By means of quantitative precipitin tests, the equivalence points of the capsular antigens from streptococcus type 87 and from

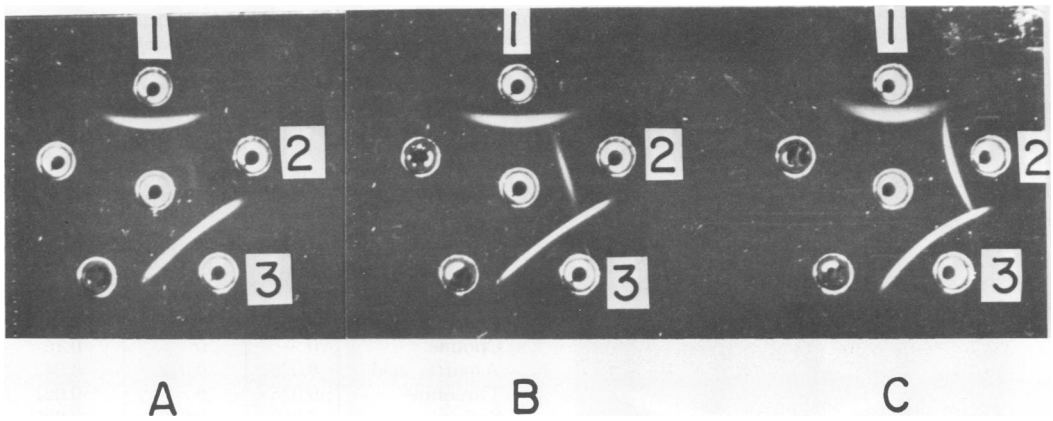

FIG. 5. *Precipitin reactions of pools IVa, IVb, and IVc from a Sephadex G-200 column (Fig. 4) in center wells A, B, and C, respectively, with rabbit antisera to: streptococcus type 87 (outer well 1), pneumococcus A66R2 (outer well 2), and pneumococcus A66R2T87S (outer well 3).*

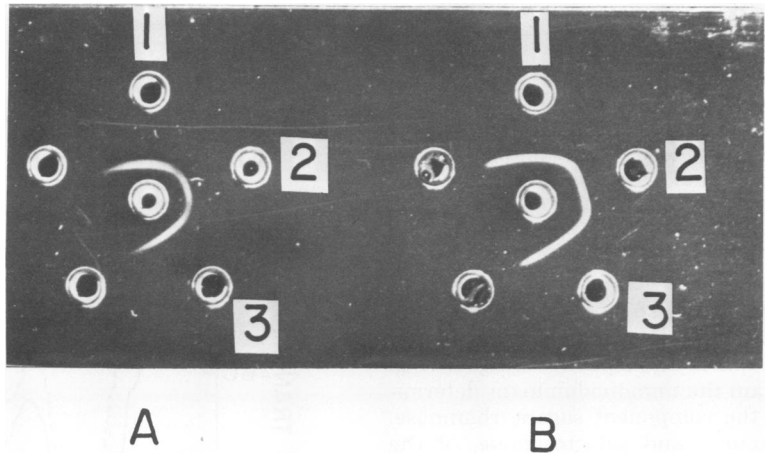

FIG. 6. *Precipitin reactions of representative polysaccharide capsular antigens from streptococcus type 87 (well 1), transformed pneumococcus A66R2T87S (well 2), and transformed pneumococcus R36NCT87S (well 3) with rabbit antisera to streptococcus type 87 (center well A) and pneumococcus A66R2T87S (center well B).*

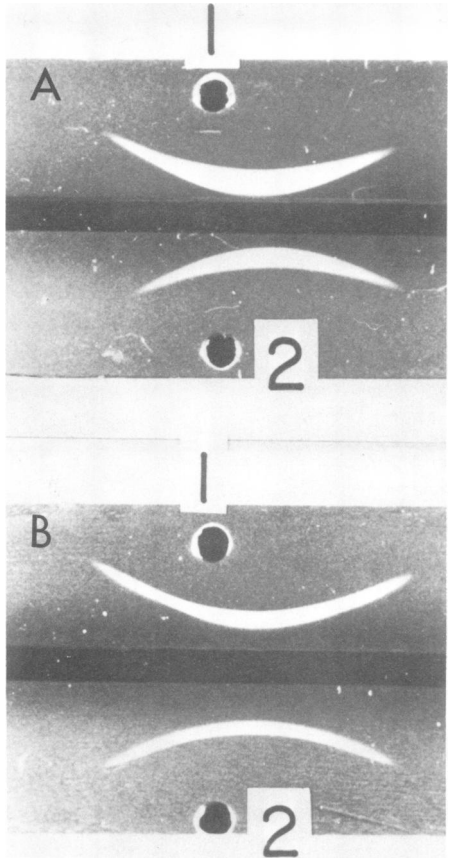

FIG. 7. *Immunoelectrophoresis of the purified capsular polysaccharide antigen of streptococcus type 87 (pool III) and of pneumococcus A66R2T87S (pool IVa) in wells 1 and 2, respectively. Troughs A and B contain antisera to the two respective organisms.*

pneumococcus A66R2T87S were determined by titrating fixed amounts of the antisera with increasing amounts of the respective antigens (Fig. 9). To ascertain the immunodominant determinant among the component sugars, rhamnose, glucose, galactose, and galactosamine, of the capsular antigens, these sugars were then tested for their ability to inhibit the precipitin reaction according to procedures detailed in the preceding report (5). Equivalent concentrations of antigen and antiserum to either the homologous or heterologous strain (Fig. 9) were used. The results of these inhibition tests with the several monosaccharides at concentrations of 10 mg/ml are given in Table 2. Only rhamnose inhibited precipitation to an appreciable degree.

DISCUSSION

The experimental data presented establish that one of the capsular components of the binary capsulated filamentous streptococcus of provisional type 87 is a polysaccharide that differs strikingly in its properties from those of the glycoprotein capsular antigen described in the accompanying report (5). The polysaccharide is composed of rhamnose, glucose, galactose, galac-

TABLE 1. *Chemical composition of the polysaccharide capsular antigens of streptococcus type 87 and of pneumococcal transformants A66R2T87S and R36NCT87S*[a]

Components	Streptococcus type 87 (pool III)	Pneumococcus A66R2T87S (pool IVa)	Pneumococcus R36NCT87S (pool I)
Rhamnose	1.00	1.00	1.00
Glucose	0.66	0.81	0.81
Galactose	1.86	1.76	1.71
Galactosamine	0.31	0.37	0.35
Phosphate	1.39	1.42	1.73
Choline	0	0	0.15
Aspartic acid	0.025	0.012	0.043
Threonine	0.025	0	0.021
Serine	0.052	0.010	0.035
Muramic acid	0	0	0.039
Glutamic acid	0.050	0	0.154
Glycine	0.063	0.014	0.047
Alanine	0.038	0.016	0.28
Valine	0.065	0.059	0
Lysine	0	0	0.130

[a] Values represent number of residues per unit of rhamnose.

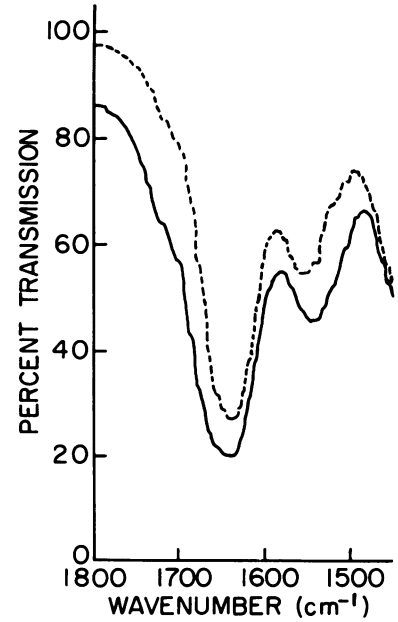

FIG. 8. *Infrared spectra of the polysaccharide antigens from streptococcus type 87* (——) *and transformed pneumococcus A66R2T87S* (------).

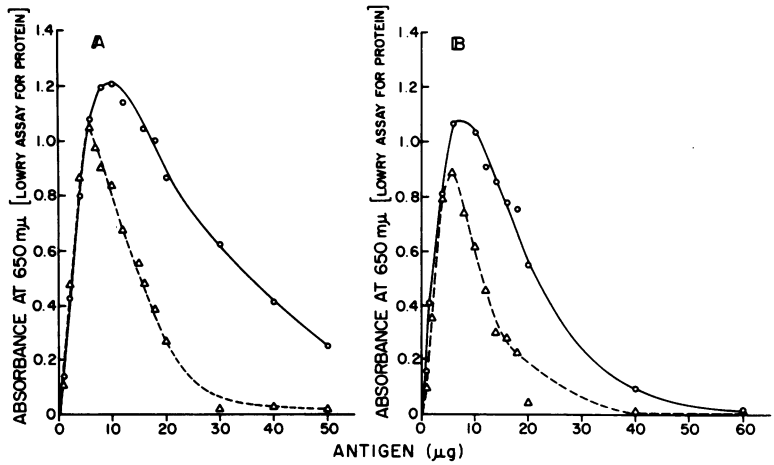

FIG. 9. *Titration curves of the polysaccharide antigens, pool III of streptococcus type 87* (○) *and pool IVa of pneumococcus A66R2T87S* (△), *with: (A) antiserum to the former and (B) antiserum to the latter.*

TABLE 2. *Inhibition of precipitation of the capsular polysaccharide antigen of streptococcus type 87 and of its pneumococcal capsular transformant A66R2T87S by component sugars of the polysaccharide*

Antigen	Antiserum to:	% Inhibition at 10-mg/ml concn of:[a]				
		GalN	GalNAc	Rham	Glc	Gal
Streptococcus type 87, pool III	Streptococcus type 87	0	0	23	2	4
Pneumococcus A66R2T87S, pool IVa	Streptococcus type 87	0	0	28	7	7
Streptococcus type 87, pool III	Transformed pneumococcus A66R2T87S	2	2	60	6	8
Pneumococcus A66R2T87S, pool IVa	Transformed pneumococcus A66R2T87S	6	9	78	11	16

[a] GalN, Galactosamine; GalNAc, *N*-acetylgalactosamine; Rham, rhamnose; Glc, glucose; Gal, galactose.

tosamine, and phosphate; rhamnose, which is the first monosaccharide to be released from the polymer by mild acid hydrolysis, appears to be its immunodominant determinant. The absence of *O*-acetyl group is indicated by the infrared spectrum of the polysaccharide antigens. The capsular polysaccharide of streptococcus type 87 can be transferred to several noncapsulated variants of pneumococcus by means of deoxyribonucleic acid-mediated transformation reactions. Immunological studies indicate that the capsular antigens produced by several strains of transformed pneumococci are closely similar to, if not identical with, that of streptococcus type 87. Simultaneous transfer of both capsular components of streptococcus type 87 to pneumococcus by means of transformation has not been observed.

ACKNOWLEDGMENTS

We thank Robert Austrian for his interest and discussions throughout the course of this work. Thanks are also due to Burton Rosan for making available the gas chromatography unit and the amino acid analyzer.

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