IMMUNOCHEMICAL STUDIES ON THE SPECIFIC CARBOHYDRATE OF GROUP G STREPTOCOCCI*, ‡

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Although Group G streptococci have been recovered frequently from various sources including the human nasopharynx and the serological classification has been firmly established (1), little attention has been directed to the distinctive immunochemical features of the group-specific carbohydrate antigen. In the present study, therefore, the Group G carbohydrate purified from isolated cell walls, has been characterized and a major chemical determinant of antigenic specificity has been identified.

In certain respects the chemical content of the cell walls and carbohydrate antigens of Group G streptococci resembles that of Groups A, A-variant, and C streptococci. The trypsinized cell walls of the latter Groups of streptococci contain two major components: a mucopeptide matrix consisting of *N*-acetylmuramic acid, *N*-acetylglucosamine, and four amino acids; and the group-specific carbohydrate, exterior to the mucopeptide, which is composed of rhamnose and *N*-acetylhexosamine (2, 3). Data to be reported here indicate that the constituent sugars of Group G carbohydrate are rhamnose, galactosamine, and galactose while the composition of the mucopeptide is similar to that of the other three groups of streptococci.

Materials and Methods

Streptococcal Strains.—Group G cell walls were prepared from strains D166B, B763A, and B549 obtained from Dr. Rebecca C. Lancefield, The Rockefeller Institute.

Preparation of Cell Walls and Group-Specific Carbohydrate.—Cell walls were prepared by previously described methods (4). The group carbohydrate was extracted from the cell walls by the formamide procedure (5).

Analytical Methods.—Analyses for rhamnose, muramic acid, glucosamine, galactosamine, and the amino acids were performed by previously described methods (3, 4).

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Galactose is frequently determined by the 3 minute Dische reaction (6), however, this procedure proved unsatisfactory for the analysis of Group G carbohydrate. During the course of this work it became clear that the rhamnose in the carbohydrate sample depressed the development of the 3 minute Dische reaction thus leading to an abnormally low analytical value for galactose. This is clearly shown in the results of an experiment depicted in Fig. 1. The 3 minute Dische test was performed on several samples of galactose to which had been added different amounts of rhamnose. It is to be noted that when the rhamnose:galactose ratio is 2:1 (the approximate ratio of these substances in the Group G carbohydrate) there is a 60 per cent depression of the optical density value from that of the galactose standard. It was thus necessary to

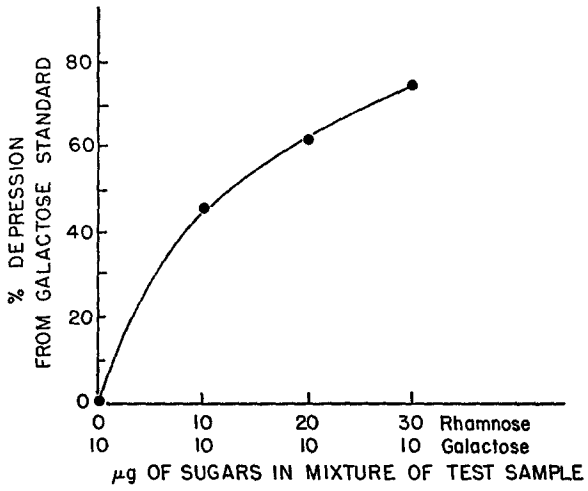

FIG. 1. Per cent depression of the color reactions of the anthrone tests for sample mixtures of rhamnose and galactose from that of the galactose standard.

measure galactose indirectly by a method which makes use of the fact that galactose and rhamnose are the only anthrone reactive sugars in the substance under investigation. In this procedure the rhamnose was first determined in one sample of carbohydrate by the 10 minute Dische reaction. Another sample was then tested by the anthrone test (7). The optical density of the test reaction for the carbohydrate is a summation due to both the rhamnose and the galactose content. The total optical density value minus that due to the rhamnose content previously determined on the first sample represents the proportion of the total optical density value due to galactose. Galactose was then calculated by comparing this value to known galactose standards. It should be noted that the intensity of the anthrone color reaction produced by mixed solutions of rhamnose and galactose standards equals the addition of the optical densities for the same amounts of rhamnose and galactose when measured in separate solutions.

Precipitin Analysis.—Quantitative precipitin analyses were performed according to the method of McCarty and Lancefield (8). Rabbit antisera were prepared by previously described methods (8).

EXPERIMENTAL

Preparation of Group-Specific Carbohydrate.—The extraction of the Group G carbohydrate from the cell walls was readily effected by the hot formamide procedure. Results of chemical analyses of the soluble carbohydrate and the mucopeptide residue for the extraction of cell walls from strain B549 are presented in Table I. The amino sugars and the amino acids identified in the residue are those characteristic of the mucopeptide of other groups of streptococci. It should be noted that although both galactosamine and glucosamine are detectable in the cell wall, only the former is identified in the carbohydrate,

TABLE I

Composition of Group G Cell Walls, the Soluble Carbohydrate, and the Insoluble Residue following Hot Formamide Extraction

	Cell walls	Formamide treatment		
		Extracted G CHO	Formamide residue	
	per cent	*per cent*	*per cent*	*mole ratio*
Rhamnose	19.6	40.7	5.5	—
Galactose	8.9	23.9	*	—
Hexosamine	8.2	—	—	—
Galactosamine	—	20.6	—	—
Glucosamine	—	0.0	10.4	2.2
Muramic acid	3.3	*	6.6	(1)
Alanine	16.9	*	24.9	10.6
Glutamic acid	7.3	*	11.1	2.9
Lysine	8.0	*	11.5	3.0
Glycine	0.9	*	0.9	0.4

G CHO, Group G carbohydrate.
* Less than 1 per cent.

while the latter is a mucopeptide component. The carbohydrate contains 40.7 per cent rhamnose, 23.7 per cent galactose, and 20 per cent galactosamine, and only traces of the elements of the mucopeptide. The results of the chemical analyses on the carbohydrate for three different strains of Group G are tabulated in Table II. The chemical composition of Group C carbohydrate is recorded for comparison.

Precipitin Inhibition with the Constituent Sugars.—Quantitative precipitin inhibition studies were undertaken to determine which of the sugars in the Group G carbohydrate was the major determinant of antigenic specificity. The similarity of chemical composition between Groups C and G carbohydrates as noted in Table II, except for the presence of galactose in the latter, suggested the possibility that galactose was a determinant of antigenic specificity. However, when galactose or galactose containing oligosaccharides such as lactose or

stachyose were added to quantitative precipitin tests, there was no appreciable inhibition. The view that galactose was not a significant feature of the Group G antigenic determinant was substantiated by the fact that this carbohydrate did not cross-react with the antisera developed against other antigens in which galactose is a determinant. For instance there was no cross-reaction with

TABLE II

Composition of Carbohydrate Extracted with Hot Formamide

	Group G strains			Group C
	D166B	B763A	B549	
	per cent	*per cent*	*per cent*	*per cent*
Rhamnose	38.7	36.8	40.7	43.0
Galactosamine	17.8	16.2	20.6	35.1
Glucosamine	—	—	—	3.9
Galactose	20.1	21.2	23.7	—

Traces of the elements of the mucopeptide were detected in each preparation.

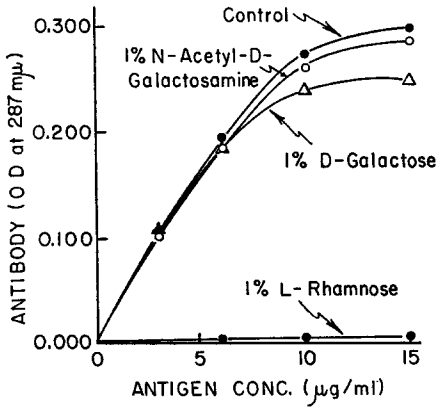

FIG. 2. Quantitative precipitin reaction between Group G carbohydrate and Group G antiserum. The final concentration of the inhibitors was 1 per cent.

Types II and XIV pneumococcal antisera or with blood group B substance antiserum.

Quantitative inhibition studies with the other constituent sugars of the G carbohydrate are depicted in Fig. 2. *N*-acetylgalactosamine was ineffective as an inhibitor of the Group G reaction, however, L-rhamnose at a final concentration of 0.5 per cent or 27.5 micromoles per ml resulted in 95 per cent inhibition at the equivalence point. Results of other precipitin inhibition studies

indicate that 50 per cent inhibition of the precipitin reaction could be achieved with as little as 3.3 micromoles of rhamnose per ml. It should be mentioned that inhibition of the precipitin reaction by rhamnose was demonstrable with each of the available Group G rabbit antisera, although all were not equally effective in this respect.

Because rhamnose demonstrated such a marked inhibitory effect on the Group G precipitin reaction, it was of special interest to perform similar experiments with various sugars which possess a steric configuration similar to

TABLE III

Inhibition of the Group G Precipitin Reaction with Various Sugars

Inhibitor	Inhibition*
	per cent
L-Rhamnose	95.5
L-Mannose	57.8
D-Fucose	23.7
L-Arabinose	21.2
D-Galactose	14.5
L-Glucose	14.5
D-Xylose	13.7
D-Arabinose	11.7
L-Xylose	11.2
D-Glucose	10.0
D-Mannose	8.3
L-Fucose	6.4

* Final concentration of inhibitor was 55 micromoles per ml. Inhibition was performed at antigen antibody equivalence.

L-rhamnose and with those that are unrelated. Results of the inhibition of the precipitin reaction are depicted in Table III. The tests were carried out at antigen-antibody equivalence and the final concentration of the inhibitor sugars was 55 micromoles per ml. Of the sugars listed other than L-rhamnose, L-mannose is the strongest inhibitor, reducing precipitation by approximately 60 per cent. By contrast D-mannose was ineffective in this respect. In the case of fucose it is interesting to note that the D form, which has a similar configuration to L-rhamnose at carbon atoms 2 and 4, is a more effective inhibitor than L-fucose, which has a configuration similar to rhamnose at carbon atoms 3 and 5. From a close examination of the findings tabulated in Table III, it is clear that there is a correlation between the effectiveness of a particular sugar as an inhibitor and its resemblance at carbon atoms 2 and 4 to the steric configuration of rhamnose.

The results of the precipitin inhibition studies provide strong evidence that L-rhamnose is a major component of the determinant of antigenic specificity. It is reasonable to assume that L-rhamnose occupies a terminal position on a side chain, however, the identity of the sugar to which it is attached and the nature of the linkage remain obscure. An enzyme with the ability to remove the determinant groups from the carbohydrate would be helpful in this respect. For instance, in establishing the importance of the terminal *N*-acetylglucosaminide residues in the serological specificity of Group A carbohydrate the initial evidence came from experiments with an induced bacterial *N*-acetyl-beta-glucosaminidase which removed the bulk of the serologically reactive terminal residues from the carbohydrate. A soil organism has been isolated which elaborates an enzyme capable of destroying the serological reactivity of the G carbohydrate; however, attempts to grow the bacteria in sufficient quantity for practical purposes have met with failure.

Relationship between Groups G- and A-Variant Carbohydrates.—Previous work has established a rhamnose oligosaccharide as the determinant grouping of Groups A-variant and C-variant carbohydrates (2, 9). A fundamental difference between the role of rhamnose in the determinant of the variant carbohydrates on the one hand and the Group G carbohydrate on the other is suggested by the fact that these carbohydrates do not cross-react with heterologous antisera. This view was substantiated further by experiments which demonstrated that variant carbohydrates have no inhibitory effect on the Group G precipitin reaction and conversely Group G carbohydrate has no inhibitory effect on the variant precipitin reactions. Convincing evidence that the rhamnose determinant of the Group G carbohydrate is unlike that of the variant carbohydrates stems from the effect of a rhamnosidase, the V enzyme identified by McCarty, on the serological activity of the antigens. Exposure of variant carbohydrates to this enzyme results in a loss of serological activity with a concomitant release of dialyzable rhamnose oligosaccharides, whereas in the case of Group G there is no alteration in the serological reactivity of the antigen and no release of dialyzable rhamnose (personal communication from M. McCarty).

DISCUSSION

In the experiments reported here attention has been directed to the chemical nature of the Group G streptococcal cell wall and to the antigenic properties of the group-specific carbohydrate. The present work is in general agreement with previous studies which have qualitatively identified the chemical constituents of Group G cell walls, although there is some discrepancy in regard to the hexose content.

Slade and Slamp (10) have identified glucose in the cell wall, a finding not reported by Cummins and Harris (11) or observed in the present study. It is conceivable that the detection of glucose in the cell walls was due to the incomplete separation, after mechanical disruption of the bacteria, of the cell walls from the protoplast membranes, a subcellular constituent known to contain glucose (12).

As with the case of Groups A and C streptococci, the hot formamide extraction procedure provides an effective means to separate the carbohydrate fraction from the insoluble mucopeptide residue. The chemical composition of the mucopeptide of Group G was essentially similar to that of Groups A and C streptococci (3, 4).

Rhamnose has been identified as a major component of the carbohydrates of Groups A, A-variant, C-variant, and C streptococci (2, 3, 9, 13), and the present findings indicate Group G is similar to the others in this respect. In the variant carbohydrates rhamnose oligosaccharides have been identified as the major determinant of antigenic specificity but in the case of Groups A and C this sugar has relatively little antigenic significance (2).

It was of particular interest therefore to observe that rhamnose markedly inhibited the Group G precipitin reaction, a finding which suggests that rhamnose monosaccharide, as the primary determinant of antigenic specificity, is terminal to the other constituent sugars in the carbohydrate. These results indicate a fundamental difference between the role of rhamnose in the determinant grouping of variant carbohydrates on the one hand and Group G on the other.

SUMMARY

Group G hemolytic streptococcal cell walls which have been treated with trypsin are composed of a group-specific polysaccharide moiety and a mucopeptide matrix. The mucopeptide contains *N*-acetylglucosamine, *N*-acetylmuramic acid, alanine, glutamic acid, lysine, and glycine, a composition similar to that of other groups of streptococci. The Group G carbohydrate is composed of rhamnose, *N*-acetylgalactosamine, and galactose. Serological studies suggest that the monosaccharide of L-rhamnose is a major component of the determinant of antigenic specificity.

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