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The α-L-(1→2)-Trirhamnopyranoside Epitope on the Group-Specific Polysaccharide of Group B Streptococci†

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A number of epitope specificities associated with the group antigen (group B polysaccharide) of group B streptococci have been identified in a polyclonal antiserum induced in rabbits by a nonencapsulated variant strain of group B streptococci. This was achieved by using a series of oligosaccharide inhibitors, obtained by both synthetic and degradative procedures, to inhibit the binding of the group B polysaccharide to the polyclonal antiserum. While the dominant epitope expressed in the antiserum was α-L-Rha*p*(1→2)α-L-Rha*p*(1→2)α-L-Rha*p*, specificities associated with α-L-Rha*p* and α-L-Rha*p*(1→3)α-D-Gal*p*(1→3)β-D-Glc*p*NAc(1→4)α-L-Rha*p* were also identified. The dominant expression of the former epitope is consistent with its terminal location on the group antigen and also with the highly branched multiantennary structure of this antigen. Antibodies specific for the α-L-trirhamnopyranoside epitope were purified by affinity chromatography, using the synthetic trisaccharide glucitol as the hapten. Oligosaccharide inhibition studies indicate that the specificity of these antibodies is identical to that of a murine monoclonal antibody induced by the same nonencapsulated strain of group B streptococci.

Group B streptococci can be differentiated from other streptococci by the presence of a group-specific polysaccharide antigen (C substance) (9–11). The group B antigen contains L-rhamnose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and D-glucitol (15, 16), and recently Michon et al. have described its entire structure (15, 16). It consists of four different oligosaccharides which are linked by one type of phosphodiester bond from O-6 of the D-glucitol residue of one oligosaccharide to O-6 of the α-D-galactopyranosyl residue of the next to form a complex highly branched multiantennary structure. When oligosaccharides lack either of the glycose constituents they are situated at the terminii of the group B antigen. Terminal α-L-rhamnopyranosyl residues are prolific on the group B antigen, and the fact that rhamnose can inhibit the binding of the group B antigen has been reported (1, 2). Having amassed a large collection of oligosaccharide fragments of the group B antigen in our studies we have now used them to define the specificity of both polyclonal and monoclonal anti-group B streptococcal antibodies. These studies resulted in the discovery of a serologically dominant epitope specific for terminal trirhamnoside domains of the group B antigen.

MATERIALS AND METHODS

Growth of organism and isolation of the group B polysaccharide. Strains 090 (type Ia) and 090R (group B) were grown as previously described (16), and the group B polysaccharide was obtained from the culture supernatant of strain 090 by previously described procedures (16).

Oligosaccharides. The oligosaccharide fragments of the group B polysaccharide were obtained by chemical and enzymatic degradation of the native polysaccharide (15) or by synthetic procedures (20, 21). Oligosaccharides containing α-L-(1→3)- or α-L-(1→4)-rhamnobiose constituents were the generous gift of A. Liptak, L. Kossuth University, Debrecen, Hungary.

Antisera. New Zealand White rabbits were immunized with formaldehyde-killed whole organisms (strain 090R) by the method of McCarty and Lancefield (14). The production of monoclonal antibody (immunoglobulin G3 [IgG3]) GBS1/18:6/D1 specific for the group B polysaccharide has been previously described (5). Ascitic fluid was produced by injecting hybridoma cells into pristane-primed BALB/c mice.

Treatment of the group B polysaccharide with Naringinase. The group B polysaccharide (10 mg) was treated with 10 U of Naringinase from *Penicillium decumbens* (EC 3.2.1.40) (Sigma, St. Louis, Mo.) as previously described (15).

Group B polysaccharide-protein conjugates. The group B polysaccharide (120 mg) was partially depolymerized with base (5 ml of 0.5 M NaOH for 30 min at 50°C) and dephosphorylated as previously described (15). Introduction of a terminal aldehyde group into the terminal D-glucitol residues of the partially depolymerized group B antigen was accomplished by controlled periodate oxidation (7). The oxidized group B polysaccharide (50 mg) was linked by reductive amination to either bovine serum albumin (BSA) (Sigma) or monomeric tetanus toxoid (TT) (obtained from a TT preparation supplied by the Institut Armand Frappier, Laval, Quebec, Canada) by a previously described method (7), and the conjugates were purified by gel filtration, using a Bio-Gel A 0.5-m (200/400 mesh) column (1.6 by 90 cm) (Bio-Rad Laboratories, Richmond, Calif.) with phosphate-buffered saline (PBS) as the eluant. The protein content of the conjugates was determined by the method of Lowry et al. (13), and their carbohydrate content was determined by the method of Dubois et al. (4), using the group B polysaccharide as the standard. The protein/polysaccharide molar

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ratio was 1:2.4 for the TT conjugate and 1:3.0 for the BSA conjugate. The purity of the conjugates was confirmed by fast protein liquid chromatography (Pharmacia), using a gel filtration column (Superose 12 HR10/30; Pharmacia).

Oligosaccharide-BSA conjugates. 5-Methoxy-carbonylpentyl-α-L-rhamnopyranoside and 5-methoxy-carbonylpentyl-2-*O*-[2-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside were prepared by previously described procedures (21) except that 5-methoxycarbonyl pentanol was substituted for the D-glucitol derivative and used in the production of the equivalent 1-*O*-D-glucitol-α-L-rhamnopyranoside and 1-*O*-D-glucitol-α-L-trirhamnopyranoside derivatives. The hydrazide derivative of the oligosaccharide methyl esters listed above was made by using hydrazine hydrate (18) in anhydrous ethanol, and following activation of the hydrazides with dinitrogen tetroxide, they were added directly to an aqueous solution of BSA to form the conjugates (18). The carbohydrate content of the conjugates was determined by the cysteine-sulfuric acid method (3) and indicated the incorporation of approximately 40 haptens per BSA molecule for both conjugates.

(1→2)-linked α-L-trirhamnopyranoside affinity column. To activate the trirhamnoside hapten, an aldehyde was selectively introduced into the terminal glucitol residue of α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→1′)-D-glucitol by controlled periodate oxidation (7). This oligosaccharide was previously prepared both by synthesis (21) and by aqueous hydrogen fluoride degradation of the group B polysaccharide (15).

The oxidized trirhamnoside (45 mg) was dissolved in water (3 ml) and reacted with ammonium acetate (500 mg) and sodium cyanoborohydride (50 mg) (19). A modified TNBS analysis (12) of the purified amino-functionalized trirhamnoside indicated >90% incorporation of the amino group. The amino-functionalized trirhamnoside (35 mg) was then dissolved in 0.1 M sodium bicarbonate buffer (5 ml), and the solution was shaken at 4°C for 16 h with 5 ml (wet gel) of Affigel 10 (Bio-Rad). The amount of trirhamnoside incorporated into the gel (5 mg of trirhamnoside per ml [wet gel]) was estimated from the amount of unreacted oligosaccharide recovered from the aqueous washings of the hapten-linked gel.

Antibody affinity purification. A column (1-cm diameter) containing the trirhamnoside-linked gel (3.5 ml) was equilibrated with PBS. Rabbit antiserum diluted 1:1 with PBS was applied to the column at a flow rate of 5 ml/h. The column was subsequently eluted with a series of buffers (A, B, C, and D) at 15 ml/h, using 5 to 10 bed volumes of each buffer. The elution profile is shown in Fig. 4, and the protein was detected by UV spectroscopy at 280 nm. Antibodies specific for terminal L-rhamnose were eluted by buffer A, which consisted of 0.2 M L-rhamnose in PBS. Unidentified protein was eluted by buffer B, which consisted of 0.1 M glycine at pH 10.5. Further elution of the column with buffer C (0.1 M glycine at pH 11.0) also produced a small amount of unidentified protein. Finally, antibodies specific for the rhamnotrioside epitope were eluted with buffer D (0.1 M glycine at pH 11.5). The specificities of the two antibody fractions eluted by buffers A and D were confirmed by enzyme-linked immunosorbent assay (ELISA) (see below), using rhamnoside- and rhamnotrioside-BSA conjugates, the preparation of which was previously described. While the antibody fraction eluted by buffer A reacted with both conjugates, that eluted by buffer D reacted only with the rhamnotrioside-BSA conjugate. None of the other fractions reacted with either conjugate.

ELISA for binding of antibody. The wells of Linbro EIA microtiter plates (Flow Laboratories, Mississauga, Ontario, Canada) were each coated with 200 ng of the group B polysaccharide or related BSA conjugates in PBS buffer for 3 h at room temperature. The plates were blocked with 1% BSA in PBS for 10 min at room temperature. The wells were then filled with 100 μl of serial 10-fold dilutions of antiserum in PBS containing 0.05% Tween and 1 mg of BSA per ml, and the plates were left for 1 h at room temperature. After the plates were washed, 50 μl of a 1:200 dilution in PBS of peroxidase-labeled goat anti-mouse or anti-rabbit IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) was added to each well. Following incubation of the plates for 1 h at room temperature the plates were washed (five times) with PBS-Tween 20, and the tetramethylbenzidine substrate (50 μl) (Kirkegaard & Perry) was added. The reaction was stopped after 15 min by the addition of 50 μl of 1 M H_3PO_4, and the optical densities were read at 450 nm with a Titertek Multiscan M.C. (Flow Laboratories, Meckenheim, Federal Republic of Germany).

ELISA for inhibition of antibody binding. Microtiter plates as described above were coated with 500 ng of the group B streptococcal polysaccharide-TT or -BSA conjugate in PBS buffer at 37°C for 1 h. The plates were blocked with 1% BSA in PBS for 10 min at room temperature and washed (four times) with PBS-Tween 20. One hundred microliters of inhibitor and 100 μl of a 5×10^{-3} dilution in PBS of immunopurified rabbit polyclonal antiserum or affinity-purified (protein A) murine monoclonal antibody (IgG3) were incubated for 1 h at room temperature. One hundred microliters of each of these solutions was then transferred to the coated wells, and the plates were incubated at room temperature for 1 h. The wells were then washed (four times) with PBS-Tween 20, and 50 μl of peroxidase-labeled goat anti-mouse IgG (1:200) or 50 μl of peroxidase-labeled goat anti-rabbit IgG (both from Kirkegaard & Perry) was added. Following incubation for 1 h at room temperature the plates were washed (five times) with PBS-Tween 20 and the remainder of the ELISA was carried out as described above.

Quantitative precipitin analyses. Quantitative precipitin analyses were carried out by the method of Kabat and Mayer (8). Aliquots (100 μl) of a 10-fold dilution in PBS of rabbit-anti group B polysaccharide-specific serum were mixed with increasing concentrations of polysaccharide in a total volume of 200 μl (adjusted with PBS). The tubes were incubated for 1 h at 37°C and then for 4 days at 4°C, after which they were centrifuged, washed twice with cold PBS, and recentrifuged, and the quantity of antibody protein in the pellets was determined by the method of Lowry et al. (13).

Inhibition of precipitation. Inhibition of precipitation experiments were performed using aliquots (100 μl) of the 10-fold-diluted rabbit antiserum described above mixed with increasing concentrations of inhibitor and allowed to stand for 1 h at 37°C. Group B polysaccharide (0.5 μg), sufficient to precipitate just less than the maximum antibody precipitated in the antigen excess zone, was then added to give a final volume of 200 μl. Quantitative precipitin analyses were then carried out as described above.

RESULTS

Inhibition of precipitation of the group B polysaccharide. Quantitative precipitation of the polyclonal anti-rabbit group B polysaccharide serum with the homologous group B polysaccharide demonstrated that it contained 1.2 mg of polysaccharide-specific antibody per milliliter. Experiments

1 α-L-Rhap 1→2 α-L-Rhap 1→2 α-L-Rhap 1→1′ D-Glucitol 3′-1 α-L-Rhap

2 α-L-Rhap 1→2 α-L-Rhap 1→2 α-L-Rhap 1→1′ D-Glucitol

3 α-L-Rhap 1→2 α-L-Rhap 1→2 α-L-Rhap OMe

4 α-L-Rhap 1→2 α-L-Rhap OMe

5 α-L-Rhap 1→2 α-L-Rhap 1→1′ D-Glucitol 3′-1 α-L-Rhap

6 α-L-Rhap 1→2 α-L-Rhap 1→2 α-L-Rhap 1→1′ D-Glucitol 3′-1 α-L-Rhap
4
↑
β-D-GlcpNAc
3
↑
α-D-Galp
3
↑
α-L-Rhap

7 α-L-Rhap 1→3 α-D-Galp 1→3 β-D-GlcpNAc 1→4 α-L-Rhap OMe

8 α-D-Galp 1→3 β-D-GlcpNAc 1→4 α-L-Rhap OMe

9 β-D-GlcpNAc 1→4 α-L-Rhap OMe

10 α-L-Rhap 1→4 α-L-Rhap 1→2 α-L-Rhap OMe

11 α-L-Rhap 1→3 α-L-Rhap OMe

12 α-L-Rhap 1→4 α-L-Rhap OMe

13 α-L-Rhap OMe

FIG. 1. Structures of oligosaccharide inhibitors. Oligosaccharide 1 was previously designated oligosaccharide III, and oligosaccharide 6 was previously designated oligosaccharide II (Fig. 8).

to inhibit the precipitation of the group B polysaccharide with this antiserum were performed using a series of oligosaccharides. These oligosaccharides (Fig. 1) were either fragments of the group B polysaccharide (15, 16) or structural homologs of them and were obtained by synthetic methods (20, 21) or by degradation of the group B polysaccharide (15, 16). The inhibition curves are shown in Fig. 2, and two distinct specificities associated with the polyclonal antiserum can be identified from these experiments. The dominant specificity is associated with terminal 1→2-linked α-L-trirhamnopyranoside, and the other is associated with terminal α-L-rhamnopyranoside. The dominance of the α-L-trirhamnopyranoside epitope can be deduced from the following data. Oligosaccharides 1, 2, and 3 are all equally good inhibitors of binding, and all contain the 1→2-linked α-L-trirhamnopyranoside epitope. It is of interest that the aglycones of these trisaccharides are relatively unimportant in their inhibitory properties despite the fact that oligosaccharides 1 and 2 contain additional features of the fragment oligosaccharides that constitute the group B polysaccharide (15).

Removal of a terminal α-L-rhamnopyranosyl residue from oligosaccharides 1 and 3 to give oligosaccharides 4 and 5 considerably reduces their inhibitory properties. Thus, the terminally located 1→2 linkage between the terminal and penultimate α-L-rhamnopyranosyl residues of the trirhamnopyranoside epitope must be important to its binding. This was also confirmed by inhibition experiments using oligosaccharides 10, 11, and 12, in which the terminal 1→2 linkage is replaced by a 1→3 or 1→4 linkage. Even changing the terminal α-L-(1→2) linkage of the entire trirhamnoside epitope as expressed in oligosaccharide 3 to an α-L-(1→4)

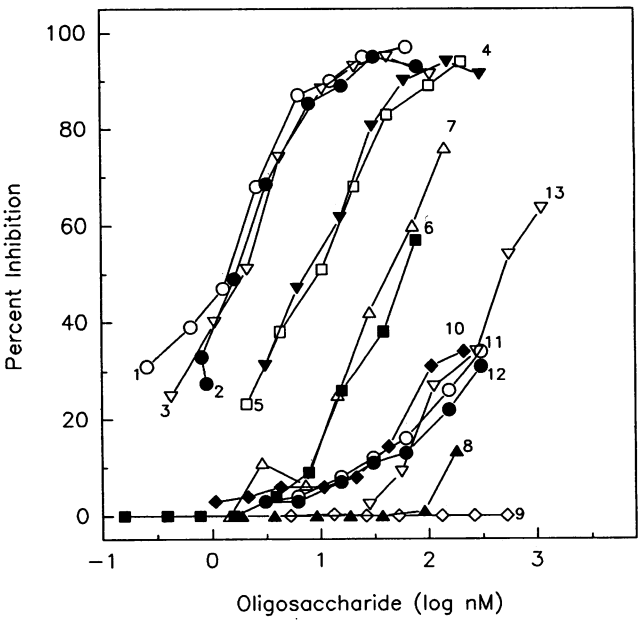

FIG. 2. Quantitative precipitation inhibition of binding of group B polysaccharide to polyclonal anti-rabbit group B polysaccharide-specific serum (induced by strain 090R), using various oligosaccharides (oligosaccharides 1 to 13). The structures of these oligosaccharides are shown in Fig. 1.

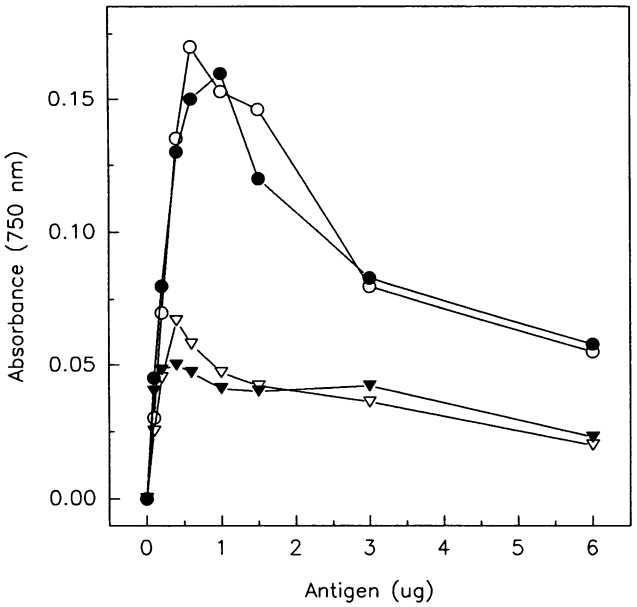

FIG. 3. Quantitative precipitin curves for reaction of native type Ia (○) and type III (●) group B polysaccharides and their respective Naringinase-treated products (▽ and ▼) with polyclonal anti-rabbit group B polysaccharide-specific serum (induced by strain 090R).

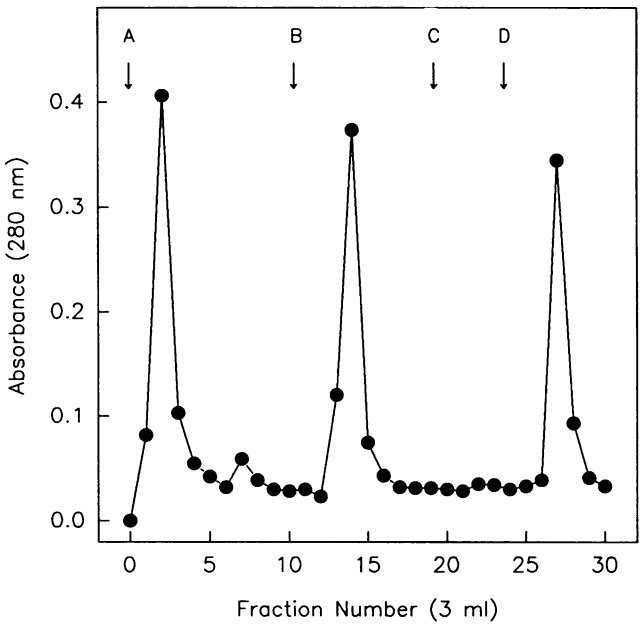

FIG. 4. Fractionation of polyclonal anti-rabbit group B polysaccharide-specific serum using a 1→2-linked-α-L-trirhamnopyranoside affinity column. Terminal α-L-rhamnopyranoside-specific antibody was eluted with buffer A, and the trirhamnoside-specific antibody was eluted with buffer D.

linkage to give oligosaccharide 10 rendered the latter oligosaccharide noninhibitory for antibodies directed against the α-L-trirhamnopyranoside epitope.

The dominance of the α-L-trirhamnopyranoside epitope in the serology of the polyclonal rabbit antiserum was confirmed by quantitative precipitation experiments in which the native group B polysaccharides from two sources and their Naringinase (α-L-rhamnosidase)-treated products were compared (Fig. 3). Naringinase is known to specifically remove terminal (1→2)-linked α-L-dirhamnopyranoside from the α-L-trirhamnopyranoside epitopes of the group B polysaccharide (15, 17). The group B polysaccharide obtained from group Ia and III streptococci gave identical precipitation curves; this was also true for their Naringinase-treated products. From the precipitin curves it can be deduced that antibodies specific for the α-L-trirhamnopyranoside epitope constitute approximately two-thirds of the total precipitated antibodies. All other antibodies specific for the group B polysaccharides, including those with a specificity for α-L-rhamnopyranoside, were precipitated by the Naringinase-treated group B polysaccharide.

The antibody population specific for the terminal α-L-rhamnopyranoside epitope is best identified in inhibition experiments (Fig. 2) using oligosaccharides 10, 11, 12, and 13, which all contain this structural feature. Other oligosaccharides used in these experiments also contain this structural feature, but their inhibitory properties are more complex because they contain additional epitopic structures. The simple monomeric nature of the α-L-rhamnopyranoside epitope is demonstrated by the fact that methyl α-L-rhamnopyranoside (oligosaccharide 13) proved to be as good an inhibitor as oligosaccharides 10, 11, and 12.

Evidence for the presence in the rabbit antiserum of antibodies associated with a third epitope is provided by the inhibitory properties of oligosaccharides 6 and 7. Although like oligosaccharides 10, 11, 12, and 13, oligosaccharide 7 also contains a terminal α-L-rhamnopyranosyl residue, it is a much better inhibitor than the aforementioned oligosaccharides. Thus, a third epitope specificity can be envisaged which involves both the terminal α-L-rhamnopyranosyl residue of oligosaccharide 7 and additional neighboring glycose components. The fact that terminal α-L-rhamnopyranose is also critical to the third epitope can be ascertained by the fact that its removal from oligosaccharide 7 renders the resultant oligosaccharide 8 completely noninhibitory. The third epitope is also present in oligosaccharide 6, which is an actual component of the group B polysaccharide.

Fractionation of the rabbit antiserum described above into epitope specificities based on the α-L-trirhamnopyranoside and α-L-rhamnopyranoside epitopes was accomplished using an affinity column in which the α-L-trirhamnopyranoside epitope was successfully coupled to a solid support. The results of the fractionation are shown in Fig. 4. The α-L-rhamnopyranoside-specific antibody was eluted from the column by using a buffer containing 0.2 M α-L-rhamnose. Further elution of the column with glycine-containing buffers of different alkalinity finally eluted the trirhamnopyranoside-specific antibodies at pH 11.5. The fractions containing the antibodies with the two different specificities were identified by monitoring the fractions by ELISA using an α-L-rhamnopyranoside conjugate and an α-L-trirhamnopyranoside conjugate as coating antigens.

ELISA inhibition experiments using the affinity-purified α-L-trirhamnopyranoside-specific antibody were carried out, and the binding curves are shown in Fig. 5. Because the group B polysaccharide did not bind to the wells of the ELISA plates, a group B polysaccharide-BSA conjugate was used as the coating antigen. All the oligosaccharides containing the terminal α-L-trirhamnopyranoside epitope (oligosaccharides 1, 2, and 3) were equally good inhibitors of binding. Although the α-L-dirhamnopyranoside (oligosaccharide 4) was a much less potent inhibitor, it was neverthe-

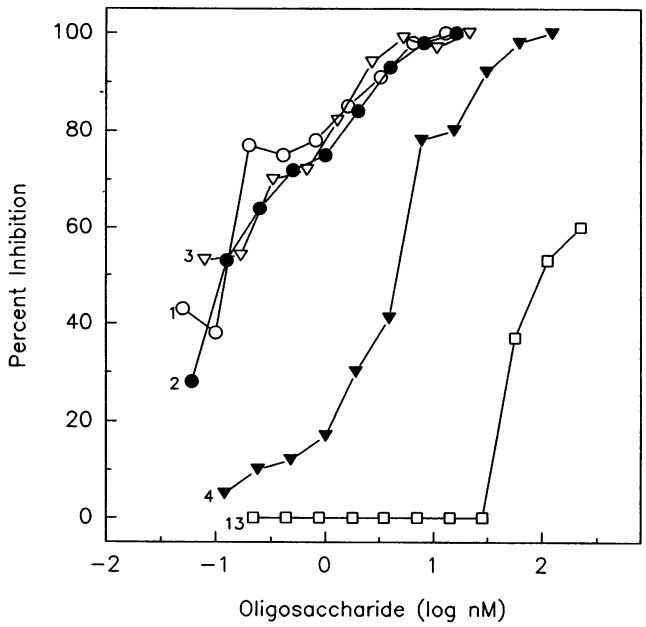

FIG. 5. ELISA inhibitions by oligosaccharides 1, 2, 3, 4, and 13 (Fig. 1) of binding of trirhamnoside affinity-purified polyclonal anti-rabbit group B polysaccharide-specific serum (induced by strain 090R) to native group B polysaccharide.

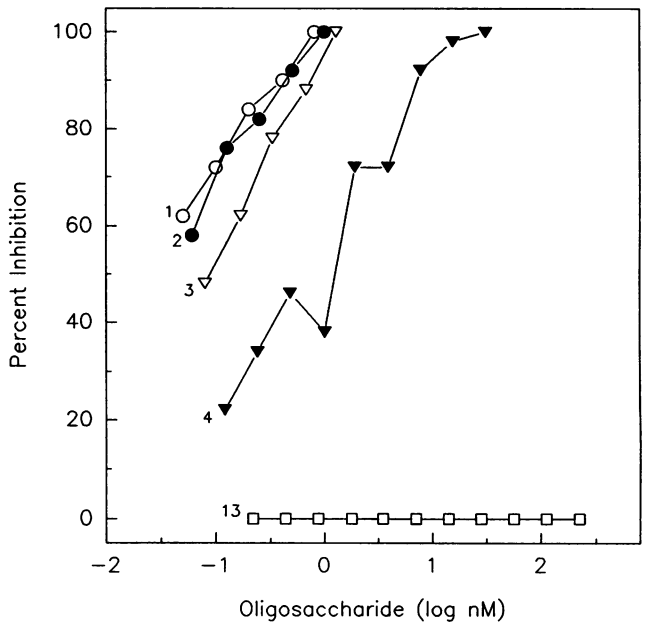

FIG. 6. ELISA inhibitions by oligosaccharides 1, 2, 3, 4, and 13 (Fig. 1) of binding of a murine monoclonal group B polysaccharide-specific antibody (induced by strain 090R) to native group B polysaccharide.

less still able to inhibit binding of the group B polysaccharide to the α-L-trirhamnopyranoside-specific antibody.

ELISA inhibition experiments using a group B polysaccharide-specific monoclonal antibody (5) and the group B polysaccharide-BSA conjugate as coating antigen gave essentially the same results as those obtained for the affinity-purified polyclonal rabbit antiserum described above. The binding curves are shown in Fig. 6. Again, all of the oligosaccharides containing the terminal α-L-trirhamnopyranoside epitope (oligosaccharides 1, 2, and 3) were good inhibitors, and the α-L-dirhamnopyranoside (oligosaccharide 4), while not as potent an inhibitor, was still able to function as an inhibitor. Methyl α-L-rhamnopyranoside (oligosaccharide 13) was also a very poor inhibitor of this system.

DISCUSSION

Group B streptococci can be defined immunologically on the basis of common epitopes. These epitopes are structural entities of the group B polysaccharide, which is present in all strains (9–11). The fine structure of the group B polysaccharide has been determined (15, 16). It is composed of four oligosaccharides, previously designated I, II, III and IV (15) (Fig. 7). These oligosaccharides are assembled in a tetraantennary structure (Fig. 8) by only one type of interoligosaccharide phosphodiester linkage between O-6 of D-glucitol and O-6 of D-galactopyranose. Because oligosaccharide III has no D-galactopyranosyl residue, it is situated at the nonreducing terminii of the tetraantennary structure. Likewise, because oligosaccharide IV is devoid of D-glucitol it is probably the oligosaccharide through which the group B antigen is linked to the cell surface of group B streptococci (15).

All the immunological results described in this paper are consistent with the proposed tetraantennary structure of the group B polysaccharide (Fig. 8). Because of its accessibility, it might be expected that α-L-rhamnopyranoside would be the immunodominant epitope; however, oligosaccharide inhibition studies of the polyclonal anti-rabbit group B streptococcal serum demonstrated that antibodies to the group B polysaccharide are dominated by those specific for the more complex trirhamnopyranoside epitope (III) as expressed in oligosaccharides 1, 2, and 3 (Fig. 1). In comparative precipitin experiments using the Naringinase-treated group B polysaccharide, it was also estimated that antibodies with the latter specificity constitute approximately 66% of the total antibody. This quantification was made possible because of the fact that only the trirhamnopyranoside epitope is removed from the group B polysaccharide by this enzyme (15, 17). Inhibition studies of affinity-purified antibodies to the trirhamnopyranose epitope indicate that while dirhamnopyranose (α-L-Rha*p*-(1→2)-α-L-Rha*p*)-containing oligosaccharides (oligosaccharides 4 and 5) do bind to a certain extent to those antibodies, high-affinity binding occurs only when the intact trirhamnopyranoside epitope is present. Monomeric methyl α-L-rhamnopyranoside (oligosaccharide 13) does not bind to antibodies expressing the trirhamnopyranoside epitope. Thus, because of the highly specific nature of this epitope and the fact that it is present on all group B streptococci, it is likely that antibodies recognizing this epitope would have potential in the diagnosis of group B streptococcal infections. It is interesting that a murine monoclonal antibody to the nonencapsulated strain (090R) of group B streptococci, used previously in the identification of group B streptococci in histopathological material (5), was not only specific for the group B polysaccharide but also recognized the same trirhamnopyranoside epitope. This was demonstrated in oligosaccharide inhibition studies in which both monoclonal and affinity-purified trirhamnopyranose-specific antibodies exhibited identical inhibition curves.

The polyclonal anti-rabbit group B streptococcal serum also contains antibodies specific for terminal α-L-rhamnopy-

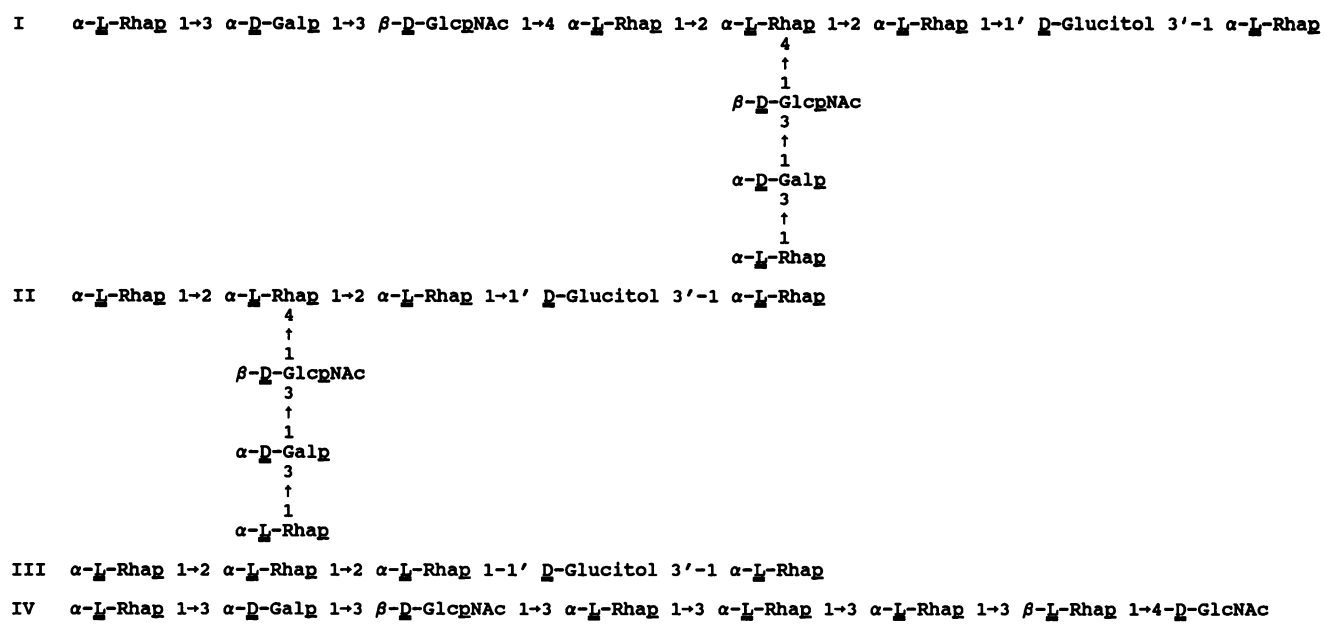

FIG. 7. Component oligosaccharides of the group B polysaccharide. The data on which the structures are based are from reference 15.

ranoside. The presence of antibodies to this epitope, a structural feature of many bacterial polysaccharides including those of other streptococci (6), would obviously reduce the specificity of the polyclonal antiserum, and therefore they were removed by affinity chromatography. If one examines the structure of the group B polysaccharide, the presence of α-L-rhamnopyranoside-specific antibody in the rabbit polyclonal antiserum is not surprising. Antibody with this specificity most likely originates from the more accessible terminal trirhamnopyranosyl units (III) of the polysaccharide (Fig. 8), although the involvement of other terminal rhamnose residues is also likely. This is because the multiantennary structure contains a total of 42 terminal α-L-rhamnopyranosyl residues inclusive of those situated on the four terminal oligosaccharides (III). In view of the abundance of terminal α-L-rhamnopyranose it is probably more surprising that the amount of antibody having this specificity is so low. Interestingly, in recent experiments in which rabbits were immunized with the same nonencapsulated strain (090R) as used in our experiments, it was found that antibodies having this specificity (α-L-rhamnopyranose) do in fact dominate the early response. The level of antibodies having this specificity then drop dramatically, resulting in a dominance of antibodies recognizing the trirhamnopyranoside epitope (unpublished results).

Antibodies with specificities other than those associated

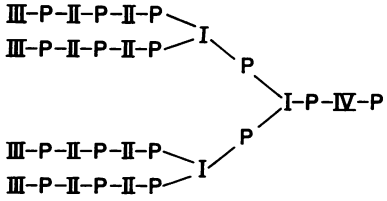

FIG. 8. Tetraantennary structure proposed for arrangement of the component oligosaccharides (I, II, III, and IV) in the group B polysaccharide.

with the trirhamnoside and monorhamnoside epitopes were also identified in the polyclonal rabbit antiserum. Oligosaccharides containing the tetrasaccharide α-L-Rha*p*(1→3)α-D-Gal*p*(1→4)β-D-Glc*p*Nac(1→4)α-L-Rha*p* were much better inhibitors of the binding of the group B polysaccharide to these antibodies than could be expected on the basis of their terminal monorhamnoside epitopes alone. The tetrasaccharide is a structural feature of oligosaccharides I and II (Fig. 7), and therefore, despite the internal location of oligosaccharides I and II in the group B polysaccharide (Fig. 8), the tetrasaccharide must still be accessible to the immune mechanism.

ACKNOWLEDGMENT

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