

Structural and Antigenic Types of Cell Wall Polysaccharides from Viridans Group Streptococci with Receptors for Oral Actinomyces and Streptococcal Lectins

JOHN O. CISAR,^{1*} ANN L. SANDBERG,¹ G. PRABHAKAR REDDY,^{2†}
CHITRANANDA ABEYGUNAWARDANA,^{2‡} AND C. ALLEN BUSH²

Oral Infection and Immunity Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892,¹ and Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, Baltimore, Maryland 21228²

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Lectin-mediated interactions between oral viridans group streptococci and actinomyces may play an important role in microbial colonization of the tooth surface. The presence of two host-like motifs, either GalNAc β 1 \rightarrow 3Gal (Gn) or Gal β 1 \rightarrow 3GalNAc (G), in the cell wall polysaccharides of five streptococcal strains accounts for the lactose-sensitive coaggregations of these bacteria with *Actinomyces naeslundii*. Three streptococcal strains which have Gn-containing polysaccharides also participate in GalNAc-sensitive coaggregations with strains of *Streptococcus gordonii* and *S. sanguis*. Each Gn- or G-containing polysaccharide is composed of a distinct phosphodiester-linked hexa- or heptasaccharide repeating unit. The occurrence of these polysaccharides on 19 additional viridans group streptococcal strains that participate in lactose-sensitive coaggregations with actinomyces was examined. Negatively charged polysaccharides that reacted with *Bauhinia purpurea* agglutinin, a Gal and GalNAc binding plant lectin, were isolated from 17 strains by anion exchange column chromatography of mutanolysin-cell wall digests. Results from nuclear magnetic resonance and immunodiffusion identified each of 16 polysaccharides as a known Gn- or G-containing structural type and one polysaccharide as a new but closely related Gn-containing type. Unlike the reactions of lectins, the cross-reactions of most rabbit antisera with these polysaccharides were correlated with structural features other than the host-like motifs. Gn-containing polysaccharides occurred primarily on the strains of *S. sanguis* and *S. oralis* while G-containing polysaccharides were more common among the strains of *S. gordonii* and *S. mitis* examined. The findings strongly support the hypothesis that lectin-mediated recognition of these streptococci by other oral bacteria depends on a family of antigenically diverse Gn- and G-containing cell wall polysaccharides, the occurrence of which may differ between streptococcal species.

Microbial colonization of the tooth surface by viridans group streptococci and actinomyces (29) is thought to involve lectin-mediated interbacterial adhesion. Lactose-sensitive coaggregations between certain streptococci and *Actinomyces naeslundii* (10, 28) and GalNAc-sensitive coaggregations between different streptococci have been observed previously (20, 23). These coaggregations are mediated by lectin-receptor interactions involving Gal- and GalNAc-reactive adhesins associated with the type 2 fimbriae of *Actinomyces naeslundii* (10), GalNAc-reactive adhesins on certain *Streptococcus sanguis* and *S. gordonii* strains (23), and complementary receptors on many other viridans group streptococcal strains (20).

The coaggregation receptors of five streptococcal strains are associated with structurally related cell wall polysaccharides, each composed of a different phosphodiester-linked hexa- or heptasaccharide repeating unit. Interestingly, the structural features of host glycoconjugates that are recognized by *A. naeslundii* type 2 fimbriae (7, 25, 36, 41) also occur within the repeating units of different streptococcal cell wall polysaccharides. Thus, GalNAc β 1-3Gal (Gn) was found in the *S. oralis* 34

(5, 24), *S. oralis* C104 (4), and *S. gordonii* 38 (32) polysaccharides, and Gal β 1 \rightarrow 3GalNAc (G) was found in the *S. mitis* J22 (2) and *S. oralis* 10557 (3) polysaccharides. Whereas the Gal- and GalNAc-reactive fimbrial lectins of *A. naeslundii* strains recognize both Gn- and G-containing receptors (14), the GalNAc-reactive adhesins of certain *S. gordonii* and *S. sanguis* strains mediate coaggregations with each of three streptococcal strains that have Gn-containing cell wall polysaccharides but not with either of two that have G-containing polysaccharides (20). Consequently, the occurrence of Gn or G receptors on viridans group streptococci may influence the interactions of these bacteria with other members of the oral microbial community.

The five strains with structurally characterized cell wall polysaccharides are members of a representative collection which includes 24 *S. sanguis*, *S. gordonii*, *S. oralis*, *S. mitis*, and *S. anginosus* strains that participate in lactose-sensitive coaggregations with *A. naeslundii* (20). Many of these streptococci also coaggregate with other strains of *S. sanguis* and *S. gordonii*. In the present investigation, cell wall polysaccharides were isolated from all but two of the remaining 19 strains with coaggregation receptors for *A. naeslundii* and identified by nuclear magnetic resonance (NMR) and antigenic criteria. The results clearly associate the coaggregation receptors of these streptococci with a family of structurally related Gn- and G-containing cell wall polysaccharides and further suggest that the occurrence of these polysaccharides may differ among streptococcal species.

* Corresponding author. Mailing address: Building 30, Room 302, NIDR, NIH, Bethesda, MD 20892. Phone: (301) 496-1822. Fax: (301) 402-0396. E-mail: jcisar@yoda.nidr.nih.gov.

† Present address: Solvay Pharmaceuticals, Atlanta, Ga.

‡ Present address: Department of Biological Chemistry, Johns Hopkins School of Medicine, Baltimore, MD 21205.

MATERIALS AND METHODS

Bacterial strains and cultivation. The source, classification, and coaggregation properties of each streptococcus have been previously described (13, 20, 21). Bacteria were cultured to stationary phase in complex medium (20) containing 0.05% Tween 80 and either 0.2 or 0.5% glucose as indicated.

Isolation of cell wall polysaccharides. Crude preparations of streptococcal cell walls were prepared from bacteria grown in complex medium containing 0.5% glucose. Streptococci were harvested by centrifugation from 6- to 10-liter stationary-phase cultures, extracted overnight in 0.1% Triton X-100 in saline, washed in phosphate-buffered saline (PBS; 0.02 M sodium phosphate, 0.15 M NaCl, 0.02% sodium azide [pH 7.2]), and digested with 1 mg of pronase (Calbiochem Corp., La Jolla, Calif.)/ml in this buffer for 5 h at 37°C. Bacteria were incubated overnight in 6 M guanidine-10 mM Tris-HCl (pH 8) to further extract soluble material. The resulting preparation of crude cell walls was washed in 10 mM sodium phosphate buffer (pH 6.5) containing 10 mM MgCl₂ and 0.04% sodium azide and suspended in this buffer (approximately 20 ml of buffer for each liter of starting bacterial culture). Polysaccharide was solubilized by overnight digestion of the cell wall suspension at 37°C with either 50 µg of mutanolysin/ml, generously provided by K. Yokogawa (46), or approximately 100 U of the commercial enzyme (Sigma Chemical Co., St. Louis, Mo.)/ml. The digest was chilled on ice prior to addition of cold trichloroacetic acid (TCA) to a final concentration of 5% (wt/vol) and immediate centrifugation at 4°C to pellet TCA-insoluble material, which was discarded. TCA-soluble material, which included the cell wall polysaccharide, was harvested, neutralized by addition of Tris, dialyzed against water, and lyophilized. Dried material, generally from 100 to 500 mg, was dissolved in 10 mM Tris-HCl (pH 7.8) containing 0.02% sodium azide (starting buffer) and applied to a column of DEAE-Sephacel (0.9 by 27 cm). The column was rinsed with at least 200 ml of starting buffer prior to elution with a 400-ml linear gradient of 0 to 200 mM NaCl prepared in starting buffer. Fractions were assayed for carbohydrate by the phenol sulfuric acid reaction (16) with glucose as standard and for *B. purpurea* agglutinin (BPA) (E-Y Laboratories Inc., San Mateo, Calif.)-reactive material by either the quantitative precipitin (14) or inhibition of hemagglutination (2) technique.

Structural characterization of cell wall polysaccharide. For ¹H NMR spectroscopy, polysaccharide samples of about 1 mg were exchanged in D₂O (99.8 atom % D) by three cycles of lyophilization and dissolved in 0.5 ml of 99.996% D₂O in a 5-mm NMR tube. Spectra were recorded on a Bruker GN-500 spectrometer at 500 MHz, and chemical shifts were determined at 24°C relative to the internal standard of acetone set at $\delta = 2.225$ ppm.

Immunological methods. Rabbit antisera against *S. oralis* 34 (R26 and R28), *S. mitis* J22 (R49 and R50), *S. oralis* 10557 (R98 and R99), *S. oralis* C104 (R100 and R101), and *S. gordonii* 38 (R102 and R103) were prepared by intravenous injections of bacteria (27, 31). Immunodiffusion experiments were performed in 1% agarose gel cast on Gel Bond Film (FMC BioProducts, Rockland, Maine). Plates were incubated overnight at 4°C to allow immunoprecipitation, soaked 1 or 2 days in cold 0.5 M NaCl with continuous agitation to remove soluble protein, rinsed briefly in water to remove salt, dried, stained with Coomassie blue by the procedure of Fairbanks et al. (17), and photographed.

Binding of radiolabeled BPA to streptococci. BPA was labeled with ¹²⁵I (100 mCi/ml; Amersham Corp., Arlington Heights, Ill.) in the presence of Iodo beads (Pierce, Rockford, Ill.). Following gel filtration and dialysis to remove free iodine, the concentration of labeled lectin was determined by the biconchonic acid protein assay reagent (Pierce) with bovine serum albumin as standard. Specific activities ranged from 1 × 10⁴ to 7 × 10⁵ cpm/µg of protein depending on the conditions of labeling. Typically, the radioactivity of labeled lectin preparations was 90% precipitable in the presence of 20% TCA and carrier protein (4 mg of bovine serum albumin per ml) and 75% reactive with an affinity gel prepared with asialofetuin.

Binding of radiolabeled BPA to streptococci was measured by the oil centrifugation technique (40) as described previously for the binding of antibodies to actinomycetes (11). Streptococci were cultured to stationary phase in complex medium containing 0.2% glucose, harvested by centrifugation, washed three times in PBS, and suspended to a turbidity of 80 Klett units, equivalent to approximately 5 × 10⁸ bacteria/ml as determined with a Petroff-Hausser bacteria counter. BPA binding curves were determined by incubating 30 µl of bacterial cell suspension (80 Klett units), either *S. oralis* 34 or *S. mitis* J22, and amounts of ¹²⁵I-BPA that ranged from 0.4 µg to 120 µg in a total volume of 0.15 ml of PBS containing 0.05% Tween 20 (PBS-Tween). Control mixtures also were set up with no bacteria. Following incubation for 30 min at room temperature with continuous mixing, triplicate 40-µl aliquots of each experimental and control mixture were layered above 300-µl cushions of dibutyl phthalate (Eastman Kodak), bis(2-ethylhexyl)phthalate (Eastman Kodak), and Tween 20 (1.3/1.0/0.001) in microtubes. Tubes were centrifuged at 12,000 × g for 3 min to pellet bacteria, thereby separating bound from free lectin. The tips of all tubes were removed for measurements of radioactivity in a gamma counter. Bound lectin was determined from the radioactivity associated with bacteria in the tips of experimental tubes following appropriate corrections for the radioactivity in control tips with no bacteria. Free BPA was calculated by subtracting the lectin bound to bacteria (*r*) from the lectin added to each corresponding reaction mixture. The average association constant (*K_a*) of BPA for each strain was estimated by the reciprocal of free lectin concentration (*c*) at half-maximal

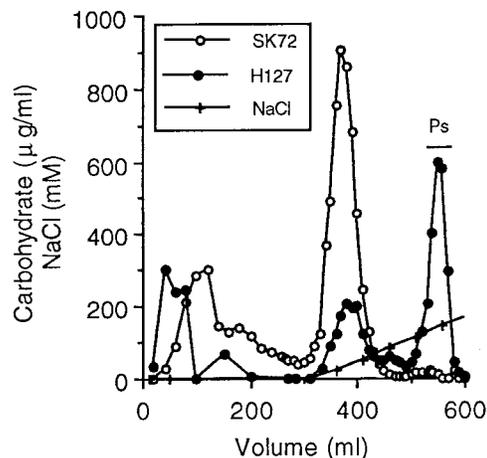


FIG. 1. Isolation of receptor polysaccharides by anion exchange column chromatography of mutanolysin digests of cell walls prepared from *S. sanguis* SK72 and *S. mitis* H127. Soluble materials from the SK72 digest (206 mg) and the H127 digest (251 mg) were applied to columns of DEAE-Sephacel in 0.01 M Tris-HCl starting buffer (pH 7.8). Each column was rinsed with starting buffer prior to elution of retained material with a linear gradient of NaCl (0 to 200 mM). Purified SK72 and H127 cell wall polysaccharides (Ps) that precipitated with BPA eluted in fractions containing from 112 to 140 mM NaCl and from 120 to 160 mM NaCl, respectively.

binding as determined from Scatchard plots of binding curves (i.e., *r/c* versus *r*). BPA concentration was calculated by using a molecular weight of 195,000 (30).

The BPA binding capacity of each strain was determined from three independent experiments each performed with bacteria from a different stationary-phase culture. Duplicate experiments were performed with the bacteria from each culture. Reaction mixtures for each experiment contained 20 µl of bacterial cell suspension (80 Klett units) and 10 µg of ¹²⁵I-BPA in a total volume of 100 µl of PBS-Tween. Bound lectin was determined as described above by using duplicate 40-µl aliquots of each reaction mixture. The results of all (i.e., six) experiments were averaged, and standard errors were calculated with *n* = 3 to reflect the variability between cultures, which was greater than the variability within each culture.

RESULTS

Isolation and structural identification of streptococcal cell wall polysaccharides. Fractionation of mutanolysin digests of cell walls by DEAE-Sephacel column chromatography resulted in three peaks of carbohydrate from 17 of the 18 *S. sanguis*, *S. gordonii*, *S. oralis*, and *S. mitis* strains examined. The first peak passed through the anion exchange column in starting buffer, another eluted at or near the beginning of the salt gradient, and the third emerged in fractions containing from 120 to 160 mM NaCl. Material that reacted with BPA, a plant lectin used previously to detect putative receptors of actinomycetes lectins (6, 14, 37), was present only in the latter fractions. Prominent peaks of BPA-reactive carbohydrate were obtained from some strains including *S. mitis* H127, and much smaller peaks were obtained from other strains including *S. sanguis* SK72 (Fig. 1). The yields, estimated from the chromatograms of these and other strains, are included in Table 1. The results obtained from fractionation of *S. oralis* 6249 cell wall digests were atypical in that peaks of either anionic carbohydrate or BPA-reactive material were not detected.

A putative receptor polysaccharide was detected but not purified from *S. anginosus* SK52. Mutanolysin-cell wall digests prepared from this strain yielded two peaks of BPA-reactive material, one that passed through DEAE-Sephacel in starting buffer and another that emerged at the beginning of the salt gradient (30 to 50 mM NaCl). The material in these peaks was

TABLE 1. Cell wall polysaccharides of viridans group streptococcal strains: yield from mutanolysin digest of cell walls, structural type, and cell surface expression measured by binding of ¹²⁵I-labeled BPA

Streptococcal strain	Yield (mg/liter) ^a	Structural type ^b	Mean (±SEM) amt (ng) of ¹²⁵ I-BPA bound ^c
<i>S. sanguis</i>			
SK72	0.2	1Gn	44 ± 12
SK75	0.1	1Gn	36 ± 8
SK77	<0.1	1Gn	59 ± 25
SK163	<0.1	1Gn	63 ± 3
SK45	0.9	1Gn	618 ± 29
SK112	1.1	1Gn	479 ± 34
<i>S. gordonii</i>			
38	2.7	2Gn	403 ± 25
SK120	0.2	3G	265 ± 11
SK121	0.3	3G	304 ± 7
<i>S. oralis</i>			
34	0.8	1Gn	192 ± 15
SK111	0.3	1Gn	357 ± 59
B2	1.7	2Gn	384 ± 42
SK92	1.6	2Gn	266 ± 9
M9811	1.6	2Gn	201 ± 4
C104	3.5	4Gn	293 ± 47
SK143	0.3	4Gn	245 ± 33
SK144	2.5	5Gn	229 ± 29
10557	2.1	3G	245 ± 33
SK23	1.4	3G	446 ± 50
6249	None	Unknown	52 ± 2
<i>S. mitis</i>			
J22	6.5	2G	488 ± 51
15914	2.2	2G	268 ± 38
H127	3.9	3G	1,370 ± 412
K103 ^d			155 ± 5
H1 ^d			8 ± 2
<i>S. anginosus</i> SK52	Not purified	Unknown	1,109 ± 68

^a Milligrams of BPA-reactive carbohydrate per liter of bacterial culture isolated by DEAE-Sephacel column chromatography of mutanolysin-digested streptococcal cell walls. Carbohydrate was estimated from phenol sulfuric acid assays of column fractions that contained BPA-reactive material with glucose as standard.

^b Structural type determined by NMR for all strains except strain SK77, which was antigenically identical to the 1Gn polysaccharide.

^c Nanograms of radiolabeled BPA bound per 40-μl reaction mixture containing 4 μg of ¹²⁵I-BPA and approximately 4 × 10⁶ streptococci. Means and standard errors of the means were calculated from experiments performed in duplicate with bacteria from three independent cultures (n = 3).

^d Strains K103 (33) and H1 (19) were included as controls. See Results for details.

not suitable for structural analysis since carbohydrate that was not BPA-reactive was also present (results not shown).

The ¹H NMR spectra recorded for 15 isolated polysaccharides (results not shown) were identical to those of previously characterized polysaccharides (2–5, 32). Comparisons of unknown and reference spectra involved the methyl group doublets of 6-deoxy sugars (~1.3 ppm), methyl group singlets of acetamido sugars (~2.0 ppm), isolated resonances between 3.3 and 3.6 ppm and between 4.0 and 4.5 ppm, general features of the crowded region between 3.6 and 4.0 ppm, chemical shifts of anomeric proton resonances (~5.5 to 4.5 ppm), and characteristic splittings resulting from H1-H2 coupling (1). Based on these ¹H structural reporter resonances, six of the isolated polysaccharides were identical to the *S. oralis* 34 polysaccharide, three were identical to the *S. gordonii* 38 polysaccharide, one was identical to the *S. oralis* C104 polysaccharide, one was identical to the *S. mitis* J22 polysaccharide, and four were identical to the *S. oralis* 10557 polysaccharide. The yield of anionic cell wall polysaccharide from *S. sanguis* SK77 was less than 0.5 mg, and consequently, no attempt was made to record the NMR spectra of this sample.

The NMR spectrum of the *S. oralis* SK144 cell wall polysaccharide was very similar to the spectrum of the previously studied *S. oralis* C104 polysaccharide (4), but a few distinct differences in the chemical shifts of structural reporter resonances were evident. A more detailed analysis showed that the C104 and SK144 structures differed only in the linkage between the α-Gal residue and ribitol (34). Whereas the C104 polysaccharide contains Galα1→1ribitol5-PO₄⁻, the SK144 polysaccharide contains Galα1→3ribitol5-PO₄⁻.

The structural types of polysaccharides isolated from different streptococci are summarized in Table 2 with designations that indicate the presence of Gn or G motifs in the repeating units of these molecules. Significantly, Gn-containing polysaccharides were isolated from all *S. sanguis* (6 of 6) and from most *S. oralis* (8 of 10) strains while G-containing polysaccharides were isolated from each of 3 *S. mitis* and from 2 of 3 *S. gordonii* strains examined (Table 1).

Antigenicity of streptococcal cell wall polysaccharides. The identification of six different cell wall polysaccharides from the streptococcal strains examined was supported by results of immunodiffusion experiments, similar to those described below, in which antigenic identity was demonstrated between the polysaccharides of each structural type isolated from different strains (results not shown). Antigenic identity was also demonstrated between the 1Gn structural type of *S. oralis* 34 and the polysaccharide of *S. sanguis* SK77, the sample that was not

TABLE 2. Structural types of cell wall polysaccharides isolated from viridans group streptococci that participate in lactose-sensitive coaggregations with *A. naeslundii*

Structural type	Polysaccharide	Streptococcus
1Gn	(→PO ₄ ⁻ →6GalNAcα1→3Rhaβ1→4Glcβ1→6Galβ1→6GalNAcβ1→3Galα1→) _n	<i>S. oralis</i> 34, SK111; <i>S. sanguis</i> SK72, SK75, SK77, SK163, SK45, SK112
2Gn	(→PO ₄ ⁻ →6GalNAcα1→3Rhaβ1→4Glcβ1→6Galβ1→6GalNAcβ1→3Galα1→) _n	<i>S. gordonii</i> 38; <i>S. oralis</i> B2, SK92, M9811
4Gn	(→1ribitol5→PO ₄ ⁻ →6Galβ1→3Galβ1→6Galβ1→6GalNAcβ1→3Galα1→) _n	<i>S. oralis</i> C104, SK143
5Gn	(→3ribitol5→PO ₄ ⁻ →6Galβ1→3Galβ1→6Galβ1→6GalNAcβ1→3Galα1→) _n	<i>S. oralis</i> SK144
2G	(→PO ₄ ⁻ →6GalNAcα1→3Rhaβ1→4Glcβ1→6Galβ1→6Galβ1→3GalNAcα1→) _n	<i>S. mitis</i> J22, ATCC 15914
3G	(→PO ₄ ⁻ →6Galα1→3Rhaβ1→4Glcβ1→3Galβ1→6Galβ1→3GalNAcα1→) _n	<i>S. oralis</i> ATCC 10557, SK23; <i>S. gordonii</i> SK120, SK121; <i>S. mitis</i> H127
	$\begin{array}{c} \text{Rha}\alpha 1 \\ \nearrow \\ \text{2} \\ \text{(OAc)}_{0.33} \end{array}$	
	$\begin{array}{c} \text{Rha}\alpha 1 \\ \nearrow \\ \text{2} \\ \text{(OAc)}_{0.33} \end{array}$	

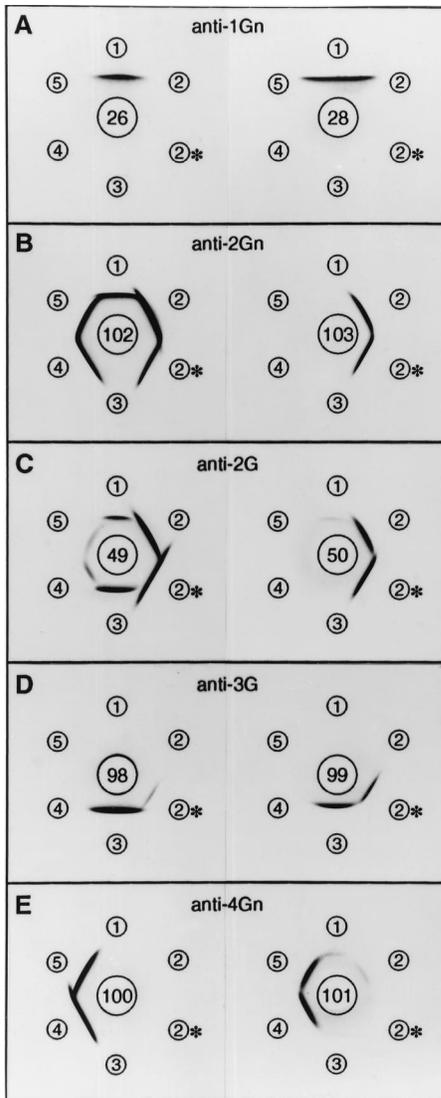


FIG. 2. Antigenic comparison of different receptor polysaccharides by immunodiffusion. (A) Anti-1Gn sera, R26 and R28 against *S. oralis* 34; (B) anti-2Gn sera, R102 and R103 against *S. gordonii* 38; (C) anti-2G sera, R49 and R50 against *S. mitis* J22; (D) anti-3G sera, R98 and R99 against *S. oralis* 10557; (E) anti-4Gn sera, R100 and R101 against *S. oralis* C104. The polysaccharide structural types isolated from the strains used for antiserum production were 1Gn (1), 2Gn (2), 2G (2*), 3G (3), 4Gn (4), and 5Gn (5).

examined by NMR. Systematic experiments were also performed to define the major cross-reactions of the different Gn- and G-containing cell wall polysaccharides (Fig. 2).

Each of two anti-1Gn sera (R26 and R28) were specific for the homologous 1Gn polysaccharide (Fig. 2A). These reactions were previously explained by the presence of a GalNAc α -containing epitope in the 1Gn polysaccharide (27). This epitope, which presumably is hidden in the 2Gn and 2G polysaccharides by the common Rha α 1 \rightarrow branches of these molecules (32), does not occur in the 3G, 4Gn, and 5Gn polysaccharides, which differ from the 1Gn polysaccharide in this region (Table 2).

One anti-2Gn serum (R103) reacted identically with the 2Gn and 2G polysaccharides and failed to react with other structural types (Fig. 2B) including the 1Gn polysaccharide, which is like the 2Gn polysaccharide but lacks Rha α 1 \rightarrow

branches (Table 2). The other anti-2Gn serum (R102) reacted with each heterologous polysaccharide except the 3G type. The repeating unit of each cross-reactive polysaccharide is similar around galactofuranose (i.e., Glc β 1 \rightarrow 6Gal β 1 \rightarrow 6 in 1Gn and 2G and Gal β 1 \rightarrow 6Gal β 1 \rightarrow 6 in 4Gn and 5Gn polysaccharides), whereas the unreactive 3G polysaccharide contains Gal β 1 \rightarrow 3Gal β 1 \rightarrow 6. In addition, Gal f of the 3G polysaccharide, but not the others, is partially O acetylated (Table 2).

The reactions of one anti-2G serum (R50) with the 2G and 2Gn polysaccharides were nearly identical and, thus, were similar to that noted above for antiserum R103. A reaction of partial identity between the 2G and 2Gn polysaccharides was noted with the other anti-2G serum (R49) (Fig. 2C), thereby indicating the detection of an antigenic difference between these isomeric G- and Gn-containing structural types (Table 2). The detection of this difference, which suggested a fraction of G-specific antibody, was consistent with the cross-reaction of antiserum R49 with the 3G polysaccharide. This antiserum also cross-reacted with the 1Gn polysaccharide and cross-reacted weakly with the other structural types.

The 2G and 3G polysaccharides were identical in their reactions with one anti-3G serum (R99) and partially identical in their reactions with the other (R98) (Fig. 2D). These antisera did not cross-react with other structural types including the 2Gn polysaccharide, which, except for the presence of Gn motifs, is like the 2G polysaccharide (Table 2).

Both anti-4Gn sera cross-reacted strongly with the 5Gn polysaccharide but weakly or not at all with other structural types (Fig. 2E). The presence of ribitol5-PO $_4^-$ \rightarrow 6Gal β 1 \rightarrow 3Gal in the repeating units of both the 4Gn and 5Gn polysaccharides distinguishes them from other structural types (Table 2). Antigenic identity between the 4Gn and 5Gn polysaccharides was noted with one antiserum (R101) but not with the other (R100). The antigenic difference detected with antiserum R100 is of interest since it can be correlated with the minor structural difference between \rightarrow 1ribitol5-PO $_4^-$ in the 4Gn polysaccharide and \rightarrow 3ribitol5-PO $_4^-$ in the 5Gn polysaccharide.

Expression of cell wall polysaccharides determined by lectin binding. The use of 125 I-labeled BPA binding to compare the cell surface expression of different G- and Gn-containing cell wall polysaccharides was assessed with *S. mitis* J22 and *S. oralis* 34, strains that possess 2G and 1Gn polysaccharides, respectively. Saturation of each strain was approached with similar amounts of added lectin (Fig. 3), and average association constants, calculated from Scatchard analysis of the binding curves (plots not shown), were approximately 1.9×10^7 liters/mol for strain J22 and 1.0×10^7 liters/mol for strain 34. Association constants similar to these were reported previously for BPA binding to human erythrocytes and lymphocytes (30). The number of BPA receptor sites per bacterial cell was approximately 10^5 for strain 34 and 4×10^5 for strain J22. These values are about 10-fold less than the number of BPA receptor sites per human erythrocyte or lymphocyte (i.e., 0.4×10^6 to 6×10^6) (30) even though the surface area of a streptococcus is at least 100-times less than those of the human cells. Thus, the average receptor density of the streptococcal surface is probably greater than that of the host cell.

The relative lectin binding capacities of different streptococcal strains varied widely (Table 1). Four strains of *S. sanguis* which yielded small amounts of cell wall polysaccharide (i.e., SK72, SK75, SK77, and SK163) bound small amounts of BPA, but four other streptococcal strains which also yielded small amounts of cell wall polysaccharide (i.e., SK120, SK121, SK111, and SK143) bound much greater amounts of BPA. Low yields of cell wall polysaccharide from the latter strains may thus reflect the amount of polysaccharide solubilized from cell

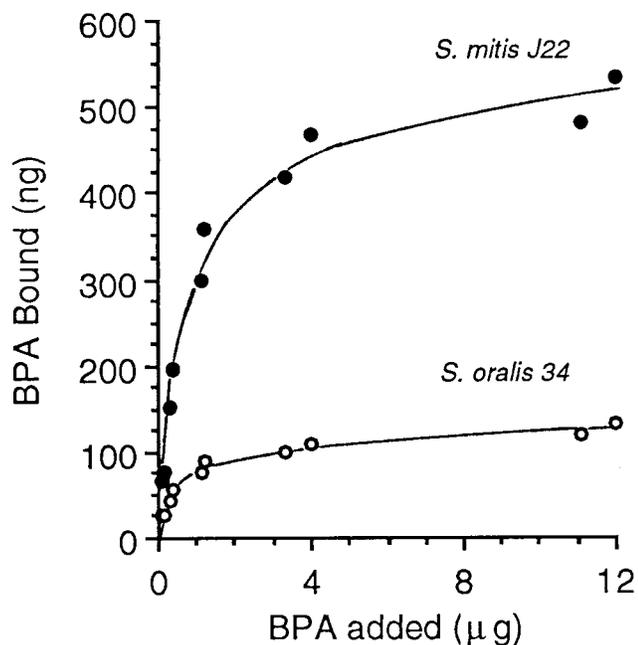


FIG. 3. Binding of ^{125}I -labeled BPA to 4×10^6 *S. oralis* 34 or *S. mitis* J22 in a total volume of 40 μl .

walls by mutanolysin digestion rather than the amount expressed on bacteria. Most streptococcal strains with coaggregation receptors bound from 200 to 400 ng of BPA per 4×10^6 bacteria (i.e., 8 μl of cell suspension adjusted to 80 Klett units) under standard assay conditions but much greater binding was observed with a few strains, most notably *S. mitis* H127. This strain bound an average of 1,370 ng of BPA, and bacteria from one of three independent cultures bound 2,100 ng of lectin. Thus, variations of up to 40-fold in BPA binding were observed between strains that expressed Gn- or G-containing cell wall polysaccharides.

In addition to the strains with Gn- and G-containing polysaccharides, two strains with cell wall polysaccharides that are not receptors of lactose-sensitive coaggregations were included as controls, *S. mitis* H1 (8, 19) and *S. mitis* K103 (33). Strain H1 bound only 8 ng of radiolabeled BPA, an amount that is within the background range of the oil centrifugation assay (i.e., 0.2% of added radioactivity) (40). Indeed, recent binding assays performed with transposon mutants of strains 34 and 15914 that make no detectable cell wall polysaccharide resulted in similar background amounts of bacteria-associated, radiolabeled lectin (45). In contrast, strain K103 bound a substantial amount of BPA (i.e., 155 ng), thereby indicating that this plant lectin reacts with streptococcal polysaccharides in addition to those that are coaggregation receptors of actinomyces lectins.

DISCUSSION

The results of the present investigation strongly suggest that specific host-like recognition motifs occur within the cell wall polysaccharides of most *S. sanguis*, *S. gordonii*, *S. oralis*, and *S. mitis* strains that participate in lactose-sensitive coaggregations with *A. naeslundii*. Thus, at least 22 of 23 representative streptococcal strains that participate in these coaggregations (20) possess either a Gn- or G-containing cell wall polysaccharide. The polysaccharides of these streptococci comprise a family that includes six structural types (i.e., 1Gn, 2Gn, 2G, 3G, 4Gn,

and 5Gn), one of which was identified in the current investigation (i.e., 5Gn) of 17 strains. Recognition of these molecules appears to depend not only on the presence of a Gn or G motif but also on other conserved features such as Gal β 1 \rightarrow 6, which may function as a hinge to expose these regions (9, 43), and the anionic phosphodiester bridge, which may interact with a subsite of the fimbrial lectin (26). It remains to be determined whether the occurrence of streptococcal receptor polysaccharides that differ antigenically between strains contributes to evasion of the secretory immune response (15). If secretory antibodies are formed against these polysaccharides during colonization it will be of considerable interest to examine the effects of such antibodies on the composition of microbial communities.

The present findings in conjunction with those of a previous study (20) also identify Gn-containing cell wall polysaccharides as the principal receptors of intrageneric coaggregation between strains of *S. sanguis*, *S. oralis*, and *S. gordonii*, species that initiate colonization of teeth or, in the case of *S. gordonii*, occur in mature dental plaque (18, 29). Previous screening of 71 streptococcal strains representative of five species for intrageneric coaggregation revealed 169 coaggregating pairs (20). Of these pairs, at least 154 (91%) include a streptococcus that has a Gn-containing polysaccharide. The other members of these pairs presumably possess GalNAc-sensitive lectins (23). The high potential for lectin-mediated interbacterial adhesion among strains of *S. sanguis*, *S. gordonii*, and *S. oralis* is suggested by the presence of GalNAc-sensitive lectins on 19 of 37 *S. sanguis* and *S. gordonii* strains and Gn-containing polysaccharides on 7 other strains of these two species and on 8 of 11 *S. oralis* strains (20). Moreover, the high frequency of Gn receptors on strains of *S. oralis* may enable this species to interact with both actinomyces and other streptococci during microbial colonization, a possibility that could contribute to the prominence of *S. oralis* in developing dental plaque (29).

Further studies are needed of two strains that did not yield purified receptor polysaccharides. One of these, *S. oralis* 6249, participates in a GalNAc-sensitive coaggregation with *S. gordonii* Challis, in addition to lactose-sensitive coaggregations with actinomyces (20), and therefore may possess Gn receptors. The failure to isolate a receptor polysaccharide from this strain may be due to a low expression of polysaccharide, as suggested by low binding of BPA to bacteria, to incomplete solubilization of cell wall polysaccharide by mutanolysin digestion, or to the occurrence of a novel receptor molecule on the strain. In contrast, BPA binding to *S. anginosus* SK52 was high, and BPA-reactive soluble material was easily detected following mutanolysin digestion of SK52 cell walls. However, soluble BPA-reactive material was not separated from other cell wall carbohydrate by anion exchange column chromatography, thereby suggesting that the putative receptor polysaccharide of strain SK52 is not composed of an oligosaccharide repeating unit that is linked by acidic phosphodiester bonds. That the first *S. anginosus* strain examined is atypical clearly emphasizes the need for further studies to purify and characterize the cell wall polysaccharides of this and related streptococcal species that occur in subgingival dental plaque (18).

Significantly, the *S. sanguis* and *S. gordonii* strains that possess Gn- and G-containing cell wall polysaccharides are negative for the Lancefield group H antigen (Blackburn) (20) as are the species *S. oralis* and *S. mitis* (21). The group H antigen has been identified as a lipoteichoic acid (35). Consequently, streptococcal strains that do not synthesize this polymer seem to synthesize cell wall polysaccharides, some of which are coaggregation receptors. The occurrence of six structural types of Gn- or G-containing cell wall polysaccharides among these

bacteria and the antigenic relationships observed between different structural types with polyclonal antisera provide a basis for the production of monoclonal antibodies that could be used to detect these molecules on bacteria in situ. Such identifications might be complicated by the detection of epitopes that do not include the host-like motifs involved in lectin recognition. Thus, antisera that detect the Rha α 1 \rightarrow branches of the 2Gn and 2G polysaccharides do not distinguish one receptor type from the other. Similarly, antibodies that react with immunodominant GalNAc α of the 1Gn repeating unit (27) or with the ribitol5-PO $_4^-$ \rightarrow 6Gal β 1 \rightarrow 3Gal regions of the 4Gn and 5Gn repeating units would not be expected to distinguish these structural types from the corresponding G-containing polysaccharides that may occur on bacteria in natural populations. This possibility clearly suggests the need for further studies to determine whether each Gn-containing structural type has a G-containing counterpart and vice versa.

In addition to epitopes that do not include the Gn or G motifs, other epitopes may overlap with these regions as suggested by the demonstration of cross-reactivity between the 2G and 3G polysaccharides. A possible structural basis for these cross-reactions is suggested by the previous identification of GalNAc as the immunodominant sugar of the 3G polysaccharide, a molecule designated as the serotype II carbohydrate in earlier studies (22, 38). Significantly, the only GalNAc unit in the 3G structure is GalNAc α in the G motif (Table 2). This GalNAc also is recognized by the GalNAc α -specific *Codium fragile* lectin which, like anti-3G antibody, precipitated with G but not Gn-containing polysaccharides (14). Anti-3G antibody of this specificity does not appear to react with host glycoconjugates (i.e., sialidase-treated erythrocytes), and consequently, it may be useful for detection of G-containing polysaccharides on bacteria in situ. The occurrence of analogous epitopes for Gn-containing polysaccharides may also permit the immunological identification of these molecules.

In addition to reducing the immunogenicity of colonizing bacteria, the host-like features of bacterial cell surface carbohydrates may play important recognition roles within the host environment. This possibility is suggested by the tendency of bacteria with polysialic acid capsules to cause meningitis (42) and also by the presence of specific lipooligosaccharide host-like structures on gonococci that occupy particular biological niches of the host environment (39, 44). Likewise, the host-like motifs expressed on oral viridans group streptococci may influence the in vivo distribution of these as well as other bacteria within oral microbial communities. Earlier observations, made from labeling human dental plaque with specific antibodies (12), suggested a close in vivo association of streptococci that possess the *S. oralis* 34 polysaccharide (i.e., 1Gn) and actinomyces with type 2 fimbriae (i.e., the structures that mediate lactose-sensitive coaggregation). The results of the present investigation provide a much improved basis for further studies to define the in vivo distribution of viridans group streptococci with Gn- or G-containing cell wall polysaccharides and also the host secretory immune response against these molecules. Information gained from such investigations is likely to provide significant insights into the recognition role of these polysaccharides in oral colonization.

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