Complete Structure of the Adhesin Receptor Polysaccharide of *Streptococcus oralis* ATCC 55229 (*Streptococcus sanguis* H1)†

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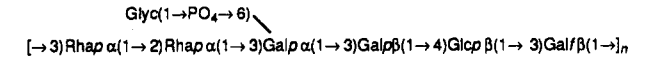
ABSTRACT: This report describes the determination of the complete primary structure of the adhesin receptor polysaccharide of *Streptococcus oralis* ATCC 55229 (previously characterized as *Streptococcus sanguis* H1), a Gram-positive bacteria implicated in dental plaque formation. The polysaccharide was isolated from *S. oralis* ATCC 55229 cells after deproteination, enzymatic hydrolysis, and ion exchange chromatography. It was shown to consist of rhamnose, galactose, glucose, glycerol, and phosphate, in molar ratios of 2:3:1:1:1. Sequence and linkage assignments of the glycosyl residues were obtained by methylation analysis followed by gas–liquid chromatography and electron-impact mass spectrometry. ^{31}P NMR spectroscopy revealed that phosphate was present in a diester, connecting glycerol to one of the galactosyl residues. High-performance liquid chromatography of a partial acid hydrolysate of the polysaccharide confirmed this finding by showing galactose 6-phosphate and glycerol 1-phosphate. The structural determination was completed by the combination of two-dimensional homonuclear Hartmann–Hahn and NOE experiments and heteronuclear $\{^1H,^{13}C\}$ and $\{^1H,^{31}P\}$ multiple-quantum coherence experiments. Thus, the adhesin receptor polysaccharide of *S. oralis* ATCC 55229 was found to be a polymer composed of hexasaccharide repeating units that contain glycerol linked through a phosphodiester to C6 of the α-galactopyranosyl residue and are joined end-to-end through galactofuranosyl-β(1→3)-rhamnopyranosyl linkages:



This structure is novel among bacterial cell surface polysaccharides in general and specifically among those implicated in dental plaque formation.

Dental plaque formation is a complicated process in which interbacterial aggregation (or coaggregation) plays a key role. The identification and biochemical characterization of the specific molecular mediators of intergeneric coaggregation has been of considerable recent interest (Abeygunawardana et al., 1991; London & Allen, 1990; Cassels et al., 1990). Responsible for intergeneric coaggregation are adhesin-to-carbohydrate receptor interactions which appear to lead to the sequential accretion of bacteria characteristic of human dental plaque. The careful examination of coaggregation mechanisms can yield valuable insight into the complex microbial ecosystem present in the human oral cavity.

The phenomenon of coaggregation was first reported by Gibbons and Nygaard (1970), who found that certain strains of plaque bacteria aggregated when mixed. Early studies identifying the molecular mediators as complimentary protein and carbohydrate receptor molecules utilized heat or protease inactivation of one of the two partner cells, as well as the use of free saccharides as inhibitors of coaggregation [McIntire et al., 1978; see the reviews of Kolenbrander (1989, 1991)]. The specific interaction between *Streptococcus oralis* ATCC 55229 [previously characterized as *Streptococcus sanguis* H1 (Kolenbrander et al., 1990)] and *Capnocytophaga ochracea* ATCC 33596 was initially described by Kolenbrander and Andersen (1984) and found to be an L-rhamnose-sensitive interaction (Weiss et al., 1987; Cassels & London, 1989). Weiss et al. (1990) identified the adhesin from *C. ochracea* ATCC 33596 specific for the *S. oralis* ATCC 55229 polysaccharide receptor as a 155-kDa outer membrane protein by utilizing coaggregation-inhibiting monoclonal antibodies. Prior studies (Cassels & London, 1989; Cassels et al., 1990) and this report identify and structurally characterize the polysaccharide and hexasaccharide repeating unit acting as the receptor for this coaggregating pair. All other characterized coaggregation polysaccharides that act as adhesin receptors in dental plaque bacteria have also been obtained from streptococci (Abeygunawardana & Bush, 1992). The receptors were isolated from *Streptococcus mitis* J22, *S. oralis* ATCC 10557, *S. oralis* 34, and *S. oralis* C104, with all involved in coaggregations with other prominent primary colonizers (*Actinomyces viscosus* and *Actinomyces naeslundii*). All four are antigenically distinct but possess a similar proposed adhesin

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[1] Abbreviations: COSY, scalar correlated spectroscopy; DIPSI-2, decoupling in the presence of scalar interactions; DQF, double quantum filtered; DSS, sodium 4,4-dimethyl-4-silapentane-1-sulfonate; 1D, 2D, one dimensional, two dimensional; EI-MS, electron-impact mass spectrometry; *f*, furanose; FID, free induction decay; GalNAc, *N*-acetylgalactosamine; GLC–MS, gas–liquid chromatography–mass spectrometry; Glyc, glycerol; HMQC, heteronuclear multiple-quantum coherence spectroscopy; HOHAHA, homonuclear Hartmann–Hahn spectroscopy; NOE, nuclear Overhauser effect; NOESY, NOE correlated spectroscopy; *p*, pyranose; PMAA, partially methylated alditol acetate; Rha, L-rhamnose; TPPI, time-proportional phase incrementation.

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receptor binding region [consisting of Galβ(1→3)GalNAc or GalNAcβ(1→3)Gal].[1]

Previous work (Cassels et al., 1990) on the cell wall polysaccharide from *S. oralis* ATCC 55229 determined the structure of a hexasaccharide repeating unit isolated by aqueous HF cleavage:

Rhap α(1→2)Rhap α(1→3)Galp α(1→3)Galp β(1→4)Glcp β(1→3)Gal
F E D C B A

In the current report, we describe the results of the structural analysis performed on the intact polysaccharide from *S. oralis* ATCC 55229.

MATERIALS AND METHODS

Bacterial Culture Conditions. *S. oralis* ATCC 55229 (*S. sanguis* H1) and *C. ochracea* ATCC 33596 were obtained from Dr. P. E. Kolenbrander (National Institute of Dental Research, Bethesda, MD). Cells were grown under anaerobic conditions in a complex medium containing tryptone, yeast extract, Tween-80, and K_2HPO_4 with 0.3% glucose (*S. oralis* ATCC 55229) (Maryanski & Wittenberger, 1975) or Schaedler broth (BBL Microbiology Systems, Cockysville, MD) (*C. ochracea* ATCC 33596).

Polysaccharide Purification. Polysaccharide was removed from *S. oralis* ATCC 55229 cell walls by treatment with mutanolysin and purified as previously described (Cassels & London, 1989), with slight modification. Briefly, intact *S. oralis* ATCC 55229 cells were sequentially treated with 0.1% Triton X-100, 0.1% Pronase (Calbiochem, San Diego, CA), and 6 M guanidine hydrochloride, with extensive washing after each treatment. This crude cell wall preparation was then digested by incubation with mutanolysin (Sigma, St. Louis, MO) for 16 h at 37 °C. After clarification, the supernatant was adjusted to a concentration of 5% TCA, and acid-precipitable material was removed by centrifugation. The supernatant was neutralized by addition of solid Tris, dialyzed extensively against deionized water, and lyophilized. Rehydrated mutanolysin extract from *S. oralis* ATCC 55229 yielded purified polysaccharide by anion-exchange chromatography on a Mono Q HR 10/10 column (Pharmacia, Piscataway, NJ). Polysaccharide was eluted at a flow rate of 2.0 mL/min with a 0.1–0.5 M NaCl gradient prepared in 2 mM Tris-HCl, pH 8.0. All fractions were monitored for presence of neutral carbohydrate by the phenol–sulfuric acid assay (Dubois et al., 1956). Neutral carbohydrate was detected in the initial column wash and eluted as a sharp peak at approximately 200–225 mM NaCl. Samples taken from carbohydrate-containing fractions were subjected to immunoelectrophoresis and developed with anti-*S. oralis* ATCC 55229 serum to determine whether the fractions contained more than one cell surface-associated antigen (Cassels & London, 1989). Multiple immune precipitin arcs appeared in the initial unbound material, while in fractions spanning the peak of salt-eluted material, each fraction contained a single antigen, and each precipitin arc was of identical mobility. The mutanolysin extract as well as the salt-eluted peak inhibited coaggregation between *S. oralis* ATCC 55229 and *C. ochracea* ATCC 33596 whole cells in a microdilution plate assay (Cassels & London, 1989). From 51.2 mg (dry weight) of mutanolysin released material, 16.8 mg of purified polysaccharide was obtained (33% yield).

Identification of Glycerol 1-Phosphate and Galactose 6-Phosphate. The *S. oralis* ATCC 55229 polysaccharide (approximately 100 μg) was subjected to partial hydrolysis in 2 M TFA (2 h, 121 °C). Excess TFA was evaporated using a stream of air, and the residue was dissolved in deionized water. Standards (1 mg/mL in deionized water) of glycerol 1-phosphate and galactose 6-phosphate (purchased from Sigma, St. Louis, MO) were also prepared. The polysaccharide hydrolysate and the standards were analyzed by HPLC using a Dionex BioLC system (Dionex, Sunnyvale, CA) with an analytical CarboPac PA1 column. Elution was by a 10–20% gradient of 1 M NaOAc in 100 mM NaOH for 20 min; then, the NaOAc concentration was increased to 50% 1 M NaOAc in 100 mM NaOH during the next 10 min. The total time for the HPLC run was 30 min, and the flow rate was 1 mL/min.

Methylation Analysis. Glycosyl linkage positions were determined by methylation analysis using the Hakomori procedure as described by York et al. (1985). Briefly, the polysaccharide was dissolved in dimethyl sulfoxide; dimethyl sulfoxide anion was added, and the sample was allowed to stir for 4 h at room temperature. An excess of iodomethane was added, and the sample was allowed to stir for 18 h at room temperature. The remaining iodomethane was evaporated using a stream of nitrogen. The permethylated polysaccharide was purified using a Sep-Pak C18 cartridge (Supelco, Bellefonte, PA), hydrolyzed in TFA, reduced with $NaBD_4$, and acetylated with acetic anhydride in pyridine. The resulting partially methylated alditol acetates (PMAAs) were analyzed by combined gas–liquid chromatography (GLC)–mass spectrometry (MS) using an HP5890/5970 GLC–MS system equipped with a 30-m SP2330 capillary column (from Supelco). The PMAAs were identified from their electron-impact (EI) mass spectra and by comparing their GLC retention times with those of authentic standards.

Nuclear Magnetic Resonance Spectroscopy. A sample (approximately 15 mg) of the cell wall polysaccharide from *S. oralis* ATCC 55229 was repeatedly dissolved in D_2O (99.99 atom% D; Cambridge Isotope Laboratories, Wilmington, DE) at room temperature and pD 6, with intermediate lyophilization. NMR spectra were recorded on Bruker AMX-600 and AM-500 spectrometers at 23 °C. ^1H chemical shifts (δ) are expressed in parts per million downfield from internal 4,4-dimethyl-4-silapentane-1-sulfonate (DSS), with an accuracy of 0.01 ppm. ^{13}C chemical shifts are expressed in parts per million downfield from internal DSS, with an accuracy of 0.02 ppm. The one-dimensional (1D) ^{31}P NMR spectra were run at pDs 6 and 10, with addition of NaOD, at 202.5 MHz on a Bruker AM-500 spectrometer; all other NMR spectra were recorded at pD 6.

Two-dimensional (2D) DQF-COSY (Piantini et al., 1982), HOHAHA (Bax & Davis, 1985), NOESY (Macura et al., 1981), HMQC (Bax et al., 1983), and HMQC-HOHAHA (Lerner & Bax, 1986) experiments were performed in the phase-sensitive mode using the time-proportional phase incrementation (TPPI) (Marion & Wüthrich, 1983) method. The HOHAHA pulse program contained a 115-ms DIPSI-2 spin-lock pulse (Rucker & Shaka, 1989). A GARP sequence (Shaka et al., 1985) was used for ^{13}C decoupling during acquisition in the $^1H\{^{13}C\}$ HMQC experiment. No decoupling was applied during the $^1H\{^{31}P\}$ HMQC experiment. For the homonuclear experiments, 512 FIDs of 4096 complex data points were collected, with 32–64 scans per FID. The spectral width was set to 4807.7 Hz (at 600 MHz) and the carrier placed at the residual HDO signal at δ 4.78. The mixing times for the NOESY and HOHAHA experiments were 80 and 54 ms, respectively. In all ^1H-detected experiments, low-power presaturation was applied to the residual HDO signal. For the $^1H\{^{13}C\}$ HMQC spectrum, 512 FIDs of 4096 complex

Table I: Glycosyl Linkages[a] of the Receptor Polysaccharide from *S. oralis* ATCC 55229 and of Its Hexasaccharide Repeating Unit

glycosyl residue	linkage position	hexasaccharide repeating unit[b]	polysaccharide
Rha*p*	terminal	1.0	0.00
	C2-linked	1.0[a]	1.0[a]
	C3-linked	0.0	0.99
Gal*p*	C3-linked	3.0	*c*
Gal*f*	C3-linked	0.0	1.85[c]
Glc*p*	C4-linked	1.0	1.16

[a] In moles relative to C2-linked Rha. [b] Taken from Cassels et al. (1990). [c] The total of C3-linked Gal*p* and Gal*f* in the polysaccharide is 1.85.

points were acquired with 64 scans per FID. The spectral width in the ^{13}C dimension was set to 75.3 ppm, with the carrier at δ 86.6, based on internal DSS. For the $^{1}H\{^{31}P\}$ HMQC data set, 128 FIDs of 2048 complex points were acquired, with the spectrometer frequency set at 202.459 MHz, δ 3.29 downfield from 85% H_3PO_4, which served as an external reference for ^{31}P chemical shifts.

NMR data were processed off-line using the FELIX software package, version 2.0 (Hare Research, Bothell, WA), on either SUN-4 or Silicon Graphics Personal Iris workstations. Data were processed typically with Lorentzian-to-Gaussian weighting functions applied in the t_2 domain and shifted squared sine-bell functions and zero-filling applied in the t_1 domain.

RESULTS

Glycosyl Linkage Position Analysis. Table I shows the positions of the glycosyl linkages of the polysaccharide of *S. oralis* ATCC 55229 as compared with those previously reported (Cassels et al., 1990) for its hexasaccharide repeating unit. The polysaccharide contains, inter alia, a C3-linked rhamnopyranosyl residue and a C3-linked galactofuranosyl residue. The PMAAs of the C3-linked Gal*p* (from residue C) and C3-linked Gal*f* (from residue A) could not be completely resolved under the GLC conditions used. However, the PMAA of C3-linked Gal*f* was readily identified by its EI mass spectrum, which showed ions of m/z 306, 277, 118, and 89.

Methylation analysis accounts for only two of the three galactosyl residues in the polysaccharide (Table I). The polysaccharide also contains phosphate, which was lost during treatment with aqueous HF (Cassels et al., 1990). This indicates that the phosphate ester group is linked to the remaining galactosyl residue (D) since the PMAA of a phosphorylated glycosyl residue would not elute from the column used during GLC analysis and, therefore, would not be detected.

Identification of Glycerol 1-Phosphate and Galactose 6-Phosphate. The presence of glycerol 1-phosphate and galactose 6-phosphate was confirmed by HPLC analysis of the TFA-hydrolyzed polysaccharide using the Dionex BioLC system (Figure 1). Two peaks were obtained with retention times identical to those of glycerol 1-phosphate and galactose 6-phosphate, respectively. Under the applied chromatographic conditions, neutral sugars eluted at the void volume of the column. The glycerol 1-phosphate peak is significantly larger than the galactose 6-phosphate peak, apparently due to preferential hydrolysis of the phosphodiester linkage between galactose and phosphate rather than the linkage between phosphate and glycerol.

NMR Spectroscopy. Complete ^{1}H and ^{13}C NMR assignments were made for the *S. oralis* ATCC 55229 polysaccharide, on the basis of values previously obtained for the isolated hexasaccharide repeating unit (Cassels et al., 1990), with additional data from HOHAHA, DQF-COSY, NOESY, and HMQC spectra. Most of the spectra strongly resemble those from the hexasaccharide; the differences are due to the presence of the galactofuranosyl-(1→3)-rhamnosyl moiety and the occurrence of glycerol phosphate attached to galactosyl residue D, as mentioned above.

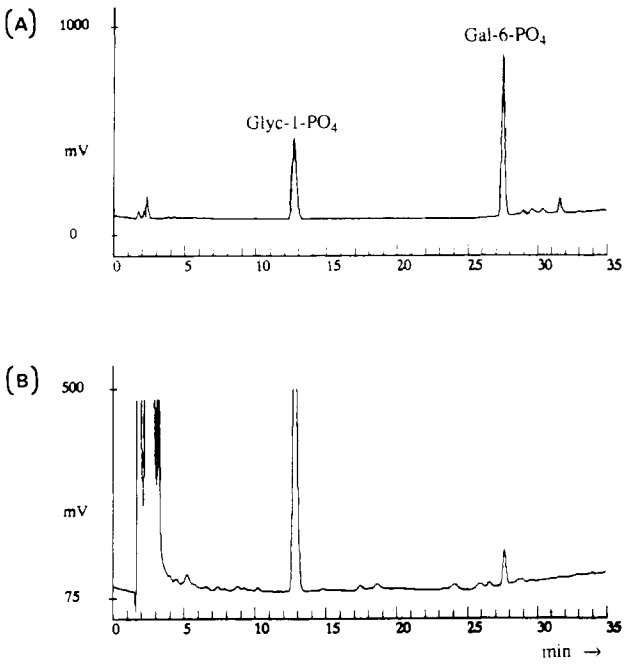

FIGURE 1: (A) HPLC chromatogram of glycerol 1-phosphate and galactose 6-phosphate standards. (B) HPLC chromatogram of the partial acid hydrolysate of the *S. oralis* ATCC 55229 polysaccharide. The conditions for chromatography on the Dionex CarboPac PA1 column are described in the text.

Figure 2A shows a region of the HOHAHA spectrum of the polysaccharide with signals identified from galactofuranosyl residue A as well as rhamnopyranosyl residue F. The ^{1}H spin pattern for residue A is clearly different from that of the reducing α,β-galactopyranose in the free hexasaccharide [compare Cassels et al., (1990)]. The corresponding ^{1}H region of the HMQC spectrum (Figure 2B), which contains the downfield signals for the ring carbons involved in glycosidic linkages, also shows the signals for Gal-A H2, H3, and H4, at chemical shifts indicative of galactofuranose (Bock & Pedersen, 1983; Bock et al., 1984)(see also Table II). The chemical shift δ 111.9 for C1 of residue A indicates that Gal*f* is in the β-configuration (Bock & Pedersen, 1983; Bock et al., 1984; Abeygunawardana & Bush, 1992). The signal for C3 of rhamnosyl residue F is found at δ 80.0 (Table III), which confirms the result of methylation analysis (see Table I) that O3 of Rha*p*-F is involved in the glycosidic linkage from residue Gal*f*-A. The presence of a strong NOESY cross-peak between H1 of Gal*f*-A and H3 of the Rha*p*-F, as seen in Figure 2C, further supports the position of the linkage.

Figure 3 shows regions of the 1D ^{1}H NMR spectrum (A), a $^{1}H\{^{31}P\}$ HMQC spectrum (B), and a $^{1}H\{^{13}C\}$ HMQC spectrum (C), all pertaining to the glycerol moiety in the polysaccharide. The signals due to the glycerol protons in spectrum A have noticeably narrower line widths than those from the glycosyl protons in the polysaccharide backbone and are also more intense in the $^{1}H\{^{13}C\}$ HMQC spectrum (C). This is consistent with the terminal position of glycerol in a side chain of the repeating hexasaccharide. A 2D HMQC-HOHAHA experiment (data not shown) confirmed that the

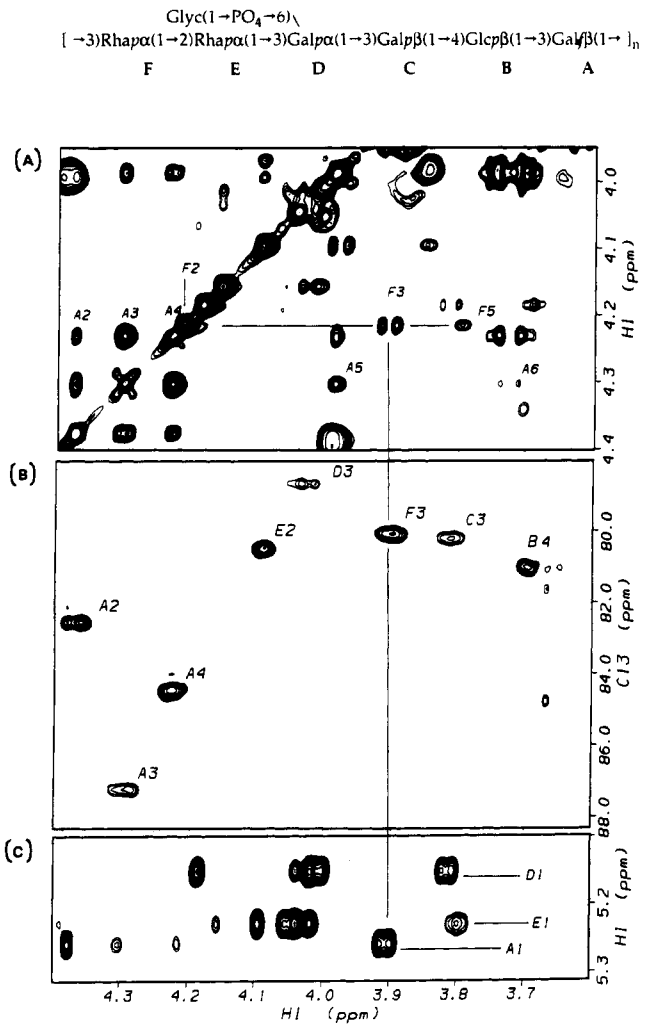

FIGURE 2: Regions of various 2D NMR spectra of *S. oralis* ATCC 55229 polysaccharide in D_2O at pD 6, recorded at 600 MHz. (A) HOHAHA spectrum (mixing time, 54 ms); (B) $^1H\{^{13}C\}$ HMQC spectrum. (C) NOESY spectrum (mixing time, 80 ms); the vertical axis covers a range of anomeric signals indicated. The occurrence of the structural entity (•→3)Gal*f*β(1→3)Rha*p*α(1→•), residues A and F, is demonstrated. The numbers in the indicated assignments refer to the carbon atoms and protons in the respective glycosyl residues.

Table II: ^1H NMR Chemical Shifts[a] for the Polysaccharide from *S. oralis* ATCC 55229

	chemical shift of						
residue	H1	H2	H3	H4	H5	H6	H6′
β-Gal*f* (A)	5.26	4.37	4.29	4.22	3.99	3.70	3.74
β-Glc*p* (B)	4.66	3.37	3.70	3.69	3.65	3.87	4.00
β-Gal*p* (C)	4.56	3.69	3.81	4.18	3.75	3.76	3.81
α-Gal*p* (D)	5.16	4.01	4.03	4.15	4.38	3.99	
α-Rha*p* (E)	5.23	4.09	3.98	3.53	3.84	1.29	
α-Rha*p* (F)	4.97	4.21	3.90	3.54	3.79	1.32	
glycerol (G)	3.87	3.90	3.61				
	3.93		3.68				

[a] Chemical shifts are referenced to internal DSS; data were acquired in D_2O at 23 °C.

Table III: ^{13}C NMR Chemical Shifts[a] for the Polysaccharide from *S. oralis* ATCC 55229

	chemical shift of					
residue	C1	C2	C3	C4	C5	C6
β-Gal*f* (A)	111.90	82.48	87.17	84.38	72.69	65.08
β-Glc*p* (B)	104.51	75.03	76.58	80.99	77.15	62.22
β-Gal*p* (C)	105.57	71.90	80.18	67.38	77.46	63.29
α-Gal*p* (D)	98.36	70.25	78.64	70.84	71.87	66.27
α-Rha*p* (E)	103.11	80.40	72.57	74.61	71.65	19.45
α-Rha*p* (F)	104.54	72.38	80.04	73.41	71.61	19.45
glycerol (G)	68.68	73.08	64.37			

[a] Chemical shifts are referenced to internal DSS; data were acquired in D_2O at 23 °C.

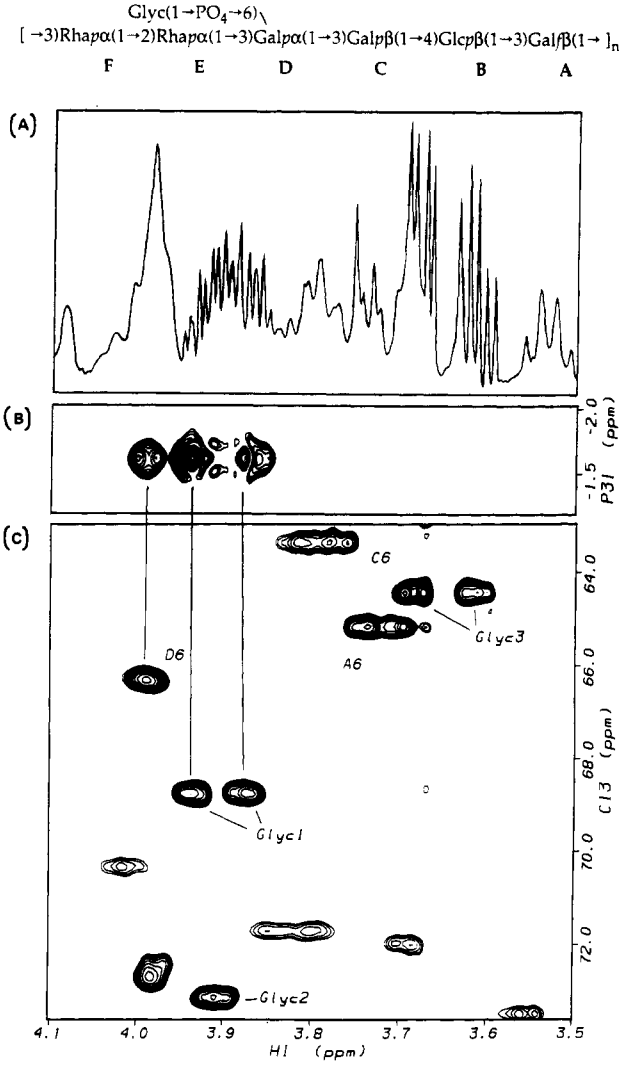

FIGURE 3: Methylene (CH_2) regions of the various NMR spectra of *S. oralis* ATCC 55229 polysaccharide in D_2O at pD 6, demonstrating the location of the phosphate diester connecting Glyc C1 and Gal-D C6. (A) 1D ^1H spectrum recorded at 600 MHz. (B) The corresponding region of the $^1H\{^{31}P\}$ HMQC spectrum, recorded at 500 MHz. (C) The corresponding region of the $^1H\{^{13}C\}$ HMQC spectrum, obtained at 600 MHz, with glycerol and some hydroxymethyl groups assigned.

signals assigned to glycerol were due to a unique spin system. The ^{31}P NMR spectrum of the polysaccharide (data not shown) gave a single signal at δ −1.7, characteristic of a phospodiester (Verkade & Quin, 1987). This signal did not shift significantly for two different pD values (6 and 10) and so confirmed the phosphate group was present as a diester, not a monoester.

A $^1H\{^{31}P\}$ HMQC spectrum (Figure 3B) provided correlations between the ^{31}P signal and three proton signals. One correlation originated from the overlapping H6,6′ protons of galactosyl residue D; the other two originated from two geminal protons of glycerol. Despite the overlap of the proton resonances from positions 1 and 2 of glycerol, the location of the phosphate group is identified as attached to C1, by virtue of the downfield shift of the glycerol C1 [δ 68.7 in Figure 3C versus δ 65 as the expected position for an unsubstituted glycerol C1 (Stortz et al., 1990)]. In comparison, the chemical

shifts of glycerol C2 and C3 at δ 73.1 and δ 64.4, respectively, are similar to those in unsubstituted glycerol (see Table III).

DISCUSSION

In the examination of the *S. oralis* ATCC 55229 polysaccharide, the presence of a phosphodiester-linked glycerol phosphate and a galactofuranosyl residue is demonstrated. Apparently, the HF treatment of the polysaccharide that produced the hexasaccharide repeating unit (Cassels et al., 1990) had cleaved the furanosidic bond between the galactofuranosyl residue A*f* and the C3-linked rhamnopyranosyl residue F, thereby converting Gal*f* into Gal*p*. Aqueous HF has been used to obtain the oligosaccharide repeating unit of other phosphorylated polysaccharides (Glaser & Burger, 1964; McIntire et al., 1988). The method was developed primarily in order to break intrachain phosphodiester linkages, freeing the oligosaccharide repeating unit. Our findings are in keeping with those of McIntire et al. (1988), who treated the polysaccharide from *S. oralis* 34 with aqueous HF (23–24 h at 2–4 °C). From that treatment, both phosphodiester linkages from the intrachain phosphodiester-linked hexasaccharide were cleaved as well as the linkage from the galactofuranose to the C6-linked *N*-acetylgalactosamine (in lower yields).

Adding the mass of glycerol phosphate to the previously determined mass of 959 Da for the HF generated hexasaccharide (Cassels et al., 1990) gives a total molecular mass of 1113 Da for the "native" hexasaccharide. The calculated phosphate percentage present in the "native" hexasaccharide is 7.2%, similar to that previously determined colorimetrically for the polysaccharide (Cassels & London, 1989).

The complete structure of the native hexasaccharide repeating unit of *S. oralis* ATCC 55229 has several similarities to the other four characterized oral streptococcal polysaccharides that participate in intergeneric coaggregation (*S. mitis* J22, *S. oralis* ATCC 10557, *S. oralis* 34, and *S. oralis* C104) (Abeygunawardana et al., 1991). For example, all contain galactofuranose and galactopyranose, all are hexasaccharides or heptasaccharides, and all contain phosphate in phosphodiester linkages; three of the four contain glucopyranose and rhamnopyranose. Important differences between the *S. oralis* ATCC 55229 and the group of four previously mentioned polysaccharides are present. All four of the non-*S. oralis* ATCC 55229 hexasaccharides are joined by phosphodiester linkages, all contain one or two residues of *N*-acetylgalactosamine, and none of the four contain glycerol (*S. oralis* C104 contains ribitol). Polysaccharides having features with a higher degree of structural similarity to *S. oralis* ATCC 55229 are found among the capsular polysaccharides of several strains of *Streptococcus pneumonia*. Several of these polysaccharides contain glycerol phosphate (on a branch or intrachain), galactofuranose, and rhamnose (Kenne & Lindberg, 1983; Robbins et al., 1983). More striking similarities to the *S. oralis* ATCC 55229 polysaccharide occur with the *S. pneumonia* type 23F (Richards & Perry, 1988) and type 11 capsular polysaccharides (Kennedy et al., 1969; Richards et al., 1985). The type 23F polysaccharide repeating unit is a tetrasaccharide containing glucopyranose, galactopyranose, and rhamnopyranose in the molar ratios 1:1:2 with a branched glycerol phosphate C3-linked to galactose. The type 11A polysaccharide repeating unit is a tetrasaccharide containing glucopyranose and galactopyranose in the ratio of 2:2 with a branched glycerol phosphate C4-linked to glucose and an acetyl group[2] on the same glucose residue as the glycerol phosphate (Kennedy et al., 1969). The type 11C repeating unit is a tetrasaccharide similar to the type 11A tetrasaccharide, containing glucopyranose, galactopyranose, and *N*-acetylglucosamine in the ratio of 1:2:1, with a branched glycerol phosphate C4-linked to *N*-acetylglucosamine and an acetyl group on the *N*-acetylglucosamine (Richards et al., 1985). These similarities are not entirely surprising since *S. oralis* is considered taxonomically close to *S. pneumonia* (Kilpper-Bälz et al., 1985). One *S. oralis* ATCC 55229 structural feature that appears among several *S. pneumonia* and group B streptococci (Kenne & Lindberg, 1983) but is not found in the other streptococcal coaggregation polysaccharides is the lactose moiety (residues C and B).

The carbohydrate receptor from *S. oralis* ATCC 55229 for the adhesin of *C. ochracea* ATCC 33596 has been the focus of this and earlier studies (Cassels & London, 1989; Cassels et al., 1990). This is the first molecular analysis of a carbohydrate receptor from a primary colonizer of the tooth surface utilized by a secondary colonizer and the first analysis of a Gram-positive receptor utilized by a Gram-negative coaggregation partner. All other oral streptococcal receptor polysaccharides characterized have been receptors for other Gram-positive primary colonizers (actinomyces). While the focus has been on this specific partner interaction, *S. oralis* ATCC 55229 has several other coaggregation partners. These partners include 14 strains of *A. naeslundii*, nine strains of *A. viscosis*, two *A. isrealii* strains, and *Rothis dentocariosa* PK 44 (Gram-positive, secondary colonizer) (Cisar et al., 1979; Kolenbrander & Andersen, 1986), while *S. oralis* ATCC 55229 does not coaggregate with other streptococci in intrageneric coaggregations (Kolenbrander et al., 1990). Of these coaggregation interactions, only the interaction with *C. ochracea* ATCC 33596 is lactose inhibitable (Kolenbrander & Andersen, 1986). Cisar et al. (1979) found that the protein (adhesin) mediator of the coaggregation was present on the streptococcal partner cell and not the actinomyces in all nine *A. viscosis* strains and all nine *A. naeslundii* strains tested that coaggregate with *S. oralis* ATCC 55229. This evidence demonstrates that *S. oralis* ATCC 55229 has at least two very distinct mechanisms useful for interbacterial networking in dental plaque distinct from those of other characterized partners.

This work and similar studies (Abeygunawardana & Bush, 1992) serve to illustrate how the complexity of dynamic interactions occurring in human dental plaque microbial interactions may be reduced to a function of protein adhesin to carbohydrate receptor recognition. A valuable insight into the structure/function relationship among complementary adhesin to carbohydrate receptor interactions is gained. With this information comes a better understanding of the strategies utilized by dental plaque bacteria for attachment and colonization into specialized ecological niches. Extensions of these studies may yield therapeutics to interrupt or reverse the accretion of dental plaque and by analogy other microbial binding events dependent on specific protein/carbohydrate interactions.

[2] It should be mentioned that some preparations of *S. oralis* ATCC 55229 polysaccharide contain a minor amount of *O*-acetyl groups. However, the acetyl groups were not abundant enough to allow us to determine their locations in the hexasaccharide.

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