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Structure and serology of the native polysaccharide antigen of type Ia group B *Streptococcus*

(capsular polysaccharide antigens/immunology of the group B *Streptococcus*/native antigens as streptococcal vaccines/sialic acid-containing antigens/structural homology with glycoproteins)

HAROLD J. JENNINGS*, KARL-GUNNAR ROSELL*, AND DENNIS L. KASPER†

*Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A OR6; and †Channing Laboratory, Harvard Medical School and the Department of Medicine, Peter Bent Brigham Hospital, Boston, Massachusetts 02115

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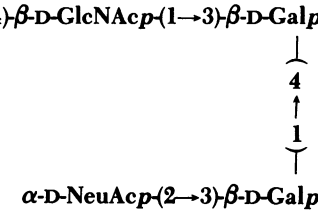
ABSTRACT The native polysaccharide antigen isolated from type Ia group B *Streptococcus* by using pH-controlled growth conditions and extraction procedures contains D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and sialic acid in the molar ratio of 2:1:1:1. The structure of the native type Ia antigen has been elucidated; it can be represented by the following repeating unit in which all the side-chain β-D-galactopyranose units are masked by sialic acid residues:



Removal of all the sialic acid groups yields the incomplete type Ia polysaccharide antigen with exposed terminal β-D-galactopyranose residues. Antisera to type Ia organisms produced in rabbits according to the Lancefield procedures contain antibodies specific for both the native and incomplete antigens. Although sialic acid is not itself a determinant in the formation of antibodies to the native polysaccharide, it is an essential part of a larger determinant. In order to maintain the high degree of immunologic specificity of the native antigen, this determinant must be at least a trisaccharide unit, because the native polysaccharide as isolated has terminal disaccharide units [α-D-NeuAcp-(2→3)-β-D-Galp] identical to those found in the human M and N blood group substances and fetuin. Formation of antibodies to the incomplete antigen is due to determinants terminating in β-D-galactopyranose residues. These determinants are probably generated by the removal of the masking sialic acid residues from the cell-associated native polysaccharide by degradative processes that occur in organisms grown without pH control.

In early immunological studies Lancefield characterized the type-specific antigens (Ia, Ib, II, and III) of group B *Streptococcus* (1–3). An additional type designated Ic was recently defined (4). The type-specific polysaccharide antigens were isolated by hot hydrochloric acid extraction of the streptococcal organisms (2, 3), and all types contain galactose, glucose, and 2-acetamido-2-deoxyglucose as exclusive components (5–10). These components occur in the molar ratio of 2:1:1, respectively, in the type Ib (8) and III (6, 10) polysaccharides. The acid-extracted antigens are immunologically incomplete and form a lower molecular weight core to the complete native antigen, which contains additional terminal acid-labile (sialic acid) residues (7–14). Thus when neutral or buffered (pH 7.0) extractions of the whole organisms are carried out the loss of sialic acid is minimized and more complete antigens can be isolated (8, 9, 12, 14, 15). However, recently it has also been established for the type III organisms that pH control during the growth of the organism is also a factor in the production and isolation of a complete native antigen (9, 10). This work also demonstrated that only the native antigen is effective for use as a human vaccine against group B streptococcal infections (9).

Further to our structural elucidation of the type III native antigen (10), we now report the structural determination of the type Ia antigen as isolated by using identical pH-controlled growth and extraction procedures (9). Like native type III antigen, and contrary to earlier reports (7), this native type Ia antigen also had no terminal galactose residues, all of them being masked by terminal sialic acid groups. Although both the type III and type Ia native polysaccharides have isomeric repeating units, their serological specificity can be attributed to strategic linkage differences. Some of these linkage differences are still retained by their respective incomplete antigens and account for the continued type of specificity of these incomplete antigens (1–4).

MATERIAL AND METHODS

Growth of the Organism. Prototype strain 090, type Ia group B *Streptococcus* was supplied by Rececca Lancefield. The lyophilized strain was rehydrated with Todd–Hewitt broth (Difco) and incubated overnight at 37°C on a blood agar plate. Strain 090 was inoculated from a blood agar plate into an erlenmyer flask containing 200 ml of Todd–Hewitt broth and incubated overnight at 37°C with stirring. This culture was transferred to 20 liters of Todd–Hewitt medium modified to contain additional glucose (14 g/liter). The organisms were grown in this medium and maintained at pH 7.5 by neutralization of acidic metabolic products with 5M NaOH by using a pH titrator (Radiometer, Copenhagen) (15, 16). All the lots were harvested at 4°C by centrifugation in a Sorvall RC-5 centrifuge, and cultures were checked to document purity at the end of the growth cycle.

Preparation of the Type Ia Core Antigen. The core type-specific polysaccharide of group B *Streptococcus* was extracted from strain 090. This strain was grown in Todd–Hewitt broth and the organisms were pelleted by centrifugation. The pelleted bacteria were suspended in 0.2 M HCl and boiled for 10 min according to the method of Lancefield (2). Debris was removed by centrifugation at 10,000 × *g* for 15 min at 4°C. The supernate was then titrated to pH 7.0 with NaOH and fractionated with 30% (vol/vol) ethyl alcohol, and the precipitate was dis-

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Abbreviations: NeuAc, *N*-acetylneuraminic acid; GC, gas/liquid chromatography; GC–MS, gas/liquid chromatography–mass spectroscopy.

carded. A crude polysaccharide was then precipitated from the extract supernate with 1.5 vol of ethyl alcohol, and after centrifugation this precipitate was saved. The supernate was then adjusted to 80% ethyl alcohol and centrifuged, and the precipitate was dissolved in 0.05 M Tris·HCl buffer, pH 7.4, and this solution was again fractionated with 1.5 vol of ethyl alcohol. The 1.5 vol alcohol precipitates were combined and extracted with cold 8% trichloroacetic acid for 4 hr at 4°C to further remove proteins and nucleic acids (12). After centrifugation at 10,000 × *g* for 10 min, the supernate was neutralized with 1 M NaOH. At this stage the supernate, which had both Ia and group B serologic reactivity in capillary precipitin tests, was chromatographed on a 2.6 × 85 cm column of Sephacryl S-200 (Pharmacia, Uppsala, Sweden) in 0.05 M Tris·HCl, pH 7.4. The serologically active fractions having type Ia or group B reactivity (3), excluding UV-absorbing material, which was of small molecular size, were combined, concentrated on a PM-30 membrane (Amicon, Lexington, MA), precipitated with 4 vol of ethyl alcohol, and suspended in 0.05 M Tris·HCl, pH 8.4. Final purification of the type Ia core antigen devoid of group B antigen was achieved on a column of DEAE-Sephacel (Pharmacia) equilibrated in 0.05 M Tris·HCl, pH 8.4.

Preparation of Type Ia (Native) Antigen. Pelleted organisms were suspended in 0.05 M phosphate buffer (pH 7.4) containing 0.15 M NaCl and 0.01 M sodium EDTA as described for type III group B *Streptococcus* (15, 16). The cell suspension was incubated at 60°C for 30 min, passed through a 25-gauge needle with manual pressure, and stirred with glass beads at 4°C for 15 hr. Washed cells were removed by centrifugation at 12,000 × *g*. Nucleic acids and proteins were partially removed by fractionation with absolute ethyl alcohol at 30% (vol/vol). The resulting supernatant was treated with 80% (vol/vol) ethyl alcohol and the precipitate was suspended in 20 ml of 0.01 M Tris·HCl buffer, pH 7.3, with 0.001 M $MgCl_2$ and 0.001 M $CaCl_2$ added. The solution was treated with DNase, RNase, and Pronase as described (15, 16). The enzyme-treated supernate was chromatographed on a 2.6 × 90 cm column of Sepharose 4B (Pharmacia) equilibrated in Tris·HCl buffer, pH 7.3. Four-milliliter fractions were collected and assayed for type Ia and group B serologic activity by capillary precipitin tests with specific antisera (2). The fractions containing only type Ia activity were pooled and concentrated to 5 ml in an ultrafiltration cell on a PM-30 membrane. Final purification took place by ion-exchange chromatography using DEAE-Sephacel (Pharmacia), equilibrated in Tris·HCl buffer, pH 8.6, and the antigen was eluted with a 0–1.0 M gradient of NaCl in this buffer. The type Ia native antigen eluted at 0.4 M NaCl and the fractions having type Ia activity were pooled, concentrated on a PM-30 membrane, and exhaustively dialyzed against distilled water. The final material was lyophilized.

Serological Methods. Rabbit antiserum to type Ia group B *Streptococcus* was made according to the method of McCarty and Lancefield (17). Immunodiffusion in agar gel was carried out by the method of Ouchterlony (18).

Instrumental Methods. Solutions were concentrated under reduced pressure below 50°C. Optical rotations were determined on a Perkin–Elmer 141 instrument with 10-mm semimicro cells at 23 ± 1°C. Gas/liquid chromatography (GC) was performed on a Hewlett–Packard 5830A instrument equipped with a flame ionization detector and a model 18850A electronic integrator. The glass columns (180 × 0.15 cm) used contained the following liquid phases (all from Supelco, Bellefonte, PA) on 100–120 mesh gas chrom Q; *i*, 3% (wt/wt) ECNSS-M at 190°C (alditol acetates) and 170°C (partially methylated alditol acetates); *ii*, 3% (wt/wt) OV-17 at 200°C (partially methylated amino-alditol acetates) and 230°C (methyl sialic acid derivatives); *iii*, (wt/wt) 3% OV-1 at 230°C (methylated trisaccharide). Combined gas/liquid chromatography–mass spectrometry (GC–MS) was carried out on a Finnigan 3100D instrument using the above columns and an ionization potential of 70 eV.

^{13}C NMR spectra were recorded in 10-mm tubes at 37°C on a Varian CFT20 spectrometer operating at 20 MHz in the pulsed Fourier-transform mode with complete proton decoupling. Chemical shifts are reported in ppm downfield from external tetramethylsilane and the ^{2}H resonance of deuterium oxide was used as a field-frequency lock signal. The polysaccharides were run as deuterium oxide solutions at concentrations of approximately 50 mg/ml.

Glycose analysis. Analyses for glycose constituents were carried out essentially by the method of Dmitriev *et al.* (19). The polysaccharide (2–3 mg) was hydrolyzed with 0.25 M sulfuric acid at 100°C for 16 hr. The hydrolysate was neutralized with barium carbonate, filtered and lyophilized. The residue in water (0.6 ml) was then treated with 33% (wt/vol) acetic acid (1 ml) and 5% aqueous sodium nitrite (1 ml) for 1 hr at room temperature. Under these conditions 2-amino-2-deoxy-D-glucose is converted to 2,5-anhydro-D-mannose (19). The solution was deionized with Dowex 50 (H^+) ion-exchange resin and lyophilized, and the residue was analyzed by GC–MS using column *i* after conversion of the glycoses into their alditol acetates (20). The D configuration was assigned to both the galactose and glucose residues by GC analysis of their (+)-2-octyl glycosides as described by Leontein *et al.* (21).

Free sialic acid was determined by the thiobarbituric acid assay method of Warren (22). The sialic acid was removed from the polysaccharide by mild acid hydrolyses (0.1 M HCl at 100°C for 10 min or 1% acetic acid at 100°C for 2 hr) or by neuraminidase (*Vibrio cholerae*) treatment (23). In this latter treatment the polysaccharide (1 mg) was treated with two separate additions of 50 units of the enzyme over a period of 72 hr as described (23).

Methylation analysis. The polysaccharides and oligosaccharides were methylated with methyl iodide in the presence of methyl sulfinyl anion according to the method of Hakomori (24). The methylated polysaccharides were recovered by dialysis against water and concentration of the aqueous solution, and the methylated oligosaccharides were recovered by chloroform extraction of an aqueous solution of the methylation mixture with subsequent concentration of the chloroform solution. The residue was dissolved in 90% (wt/vol) formic acid and heated at 100°C for 2 hr and then concentrated to dryness. This residue was further hydrolyzed with 0.25 M sulfuric acid at 100°C for 16 hr, and the hydrolysate was neutralized (barium carbonate), filtered, and reduced with $NaBH_4$. The resultant partially methylated glycitols were converted to their alditol acetate derivatives and analyzed by GC–MS (25, 26) using columns *i* and *ii*. The same method of analysis using column *ii* could also be used to identify the methylated sialic acid residue (27). In this analysis the methylated residue was first isolated as its methyl ester methyl glycoside derivative by methanolysis of the permethylated polysaccharide (27).

RESULTS

Structure of the core type Ia antigen

Like the type Ib (8), type II (5), and type III (6, 9, 10) acid-extracted incomplete antigens of group B *Streptococcus*, the incomplete antigen of type Ia also contains galactose, glucose, and 2-acetamido-2-deoxyglucose constituents (7). This was confirmed by GC–MS analysis of the alditol acetate derivatives of the hydrolysis products of the incomplete Ia antigen. This

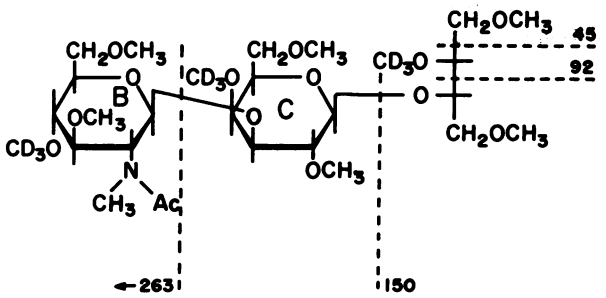

FIG. 1. Permethylated trisaccharide alditol from the incomplete type Ia antigen obtained after Smith degradation, methylation, mild acid hydrolysis, and remethylation with trideuteriomethyl iodide (D, deuterium). Some characteristic fragments in its mass spectrum are shown by the broken lines.

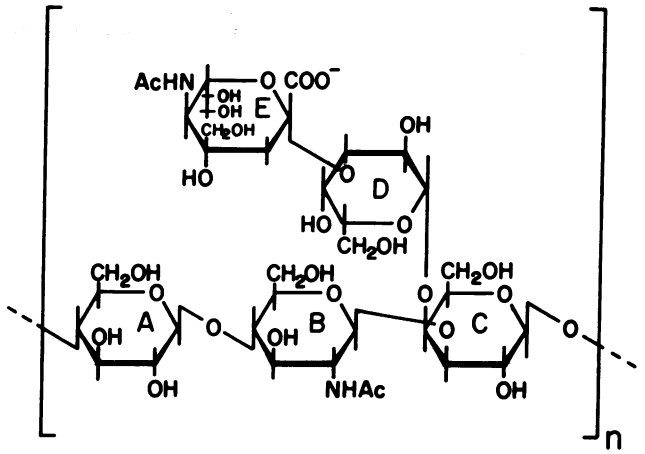

FIG. 2. Repeating unit of the native type Ia polysaccharide antigen of group B *Streptococcus*.

analysis also indicated that the above constituents were present in the molar ratio of 2:1:1, respectively. Thus the incomplete type Ia antigen has identical components in an identical molar ratio to both the incomplete type Ib (8) and type III (6, 9, 10) antigens of group B *Streptococcus*. The D configuration was assigned to the galactose and glucose constituents by virtue of the characteristic retention times of their (+)-2-octanol glycosides (21). The D configuration of the remaining 2-acetamido-2-deoxyglucose constituent can be deduced from the low value of the optical rotation ($[\alpha]_D = -4°$ in water) of the Ia antigen and the known β configuration of all its constituent sugars. This was determined from the ^{13}C NMR spectrum of the Ia antigen, which gave only one narrow signal in the region of the spectrum associated with signals of anomeric carbons (22) at 104.0 ppm. Thus, as in the incomplete type III antigen, all four sugar components (A, B, C, and D in Fig. 2) must give coincident signals whose chemical shift is consistent with their all having the β-D configuration (10).

The incomplete type Ia antigen was permethylated and on subsequent hydrolysis yielded a number of partially methylated sugars, which were identified by GC–MS (25, 26) and quantitated by GC analysis as their alditol acetate derivatives. The individual partially methylated sugars generated in this analysis and their respective mole ratios are shown in Table 1. The methylation analysis indicates the presence of one terminal D-galactopyranose residue, interchain D-glucopyranose and 2-acetamido-2-deoxy-D-glucopyranose residues both linked in their O-4 positions, and one branched D-galactopyranose residue linked at O-3 and O-4. The sequence of the individual sugar residues was elucidated by using a modification of the Smith degradation procedure (28). In this degradation the polyalcohol obtained after periodate oxidation and sodium borohydride reduction of the polysaccharide was methylated and the permethylated product was partially hydrolyzed. This yielded a partially methylated oligosaccharide, which was further methylated with tridueteriomethyl iodide. By this procedure labeled methyl groups were introduced at exposed hydroxyl groups, some of which represent linkage positions in the original polysaccharide. The resultant oligosaccharide shown in Fig. 1 was subjected to GC–MS analysis, and the mass spectrum showed the following major fragments: mass-to-charge ratio *m/e* 45, 71, 74, 92, 101, 104, 111, 115, 150, 210, 231, and 263. The sequence of the sugars in the methylated oligosaccharide (Fig. 1) was elucidated by the masses 263 (B) and 150 (C); the erythritol residue of the oligosaccharide was derived from the original glucopyranose residue (A) of the type Ia antigen. This evidence established the sequence of the linear portion of the repeating unit of the type Ia antigen shown in Fig. 2. Hydrolysis of the methylated oligosaccharide (Fig. 1) gave the individual methylated sugars (B and C), which were analyzed as their alditol acetate derivatives by using sodium borodeuteride to label C-1 (anomeric position) of B and C. The detection of 2,4,6-tri-*O*-methyl-D-galactose with a trideuteriomethyl group at O-4 indicated that the terminal D-galactopyranose residue (D) of the original type I polysaccharide was linked to O-4 of the branched D-galactopyranose residue (C), the latter residue thus having its interchain linkage at O-3 as

Table 1. Methylation analysis of the native and incomplete core type Ia streptococcal polysaccharides

Methylated glycose derivative	Relative retention time*	Molar ratios: Incomplete core polysaccharide	Molar ratios: Native polysaccharide
2,3,4,6-Tetra-*O*-methyl-D-galactose†	1.25	0.8	–
2,4,6-Tri-*O*-methyl-D-galactose†	2.28	–	0.8
2,3,6-Tri-*O*-methyl-D-glucose†	2.50	1.0	1.0
2,6-Di-*O*-methyl-D-galactose†	3.65	0.9	0.9
3,6-Di-*O*-methyl-*N*-methyl-*N*-acetyl-D-glucosamine‡	–	+	+
4,7,8,9-Tetra-*O*-methyl-*N*-methyl-*N*-acetyl-D-neuraminic acid§	–	–	+

Note: +, Slight nonquantitative response: –, not detected.

* Retention time of the alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on column *i* at 170°C.

† Identified and quantitated as the alditol acetate.

‡ Identified as its alditol acetate on column *ii* at 200°C.

§ Identified as its methyl ester methyl glycoside on column *iii* at 230°C.

determined from earlier methylation data. Also, the detection of 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucose and 1,3,4-tri-*O*-methyl-D-erythritol with trideuteriomethyl groups at O-4 and O-3, respectively, confirms the interchain linkages assigned to the C and A residues from earlier methylation data.

Structure of the native type Ia antigen

Sialic acid has been previously identified as the additional component of the native type Ia antigen (7, 12, 13), and its end-group position in the polysaccharide has been proposed (7). This is consistent with our results, which indicate that the native type Ia antigen is composed of D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and sialic acid in the molar ratio of 2:1:1:1. Neuraminidase treatment of the native type Ia antigen yielded 30% sialic acid (molar ratio 0.97), which is consistent with the polysaccharide containing one residue of sialic acid (E) per repeating unit (Fig. 2). The neuraminidase sensitivity of the antigen indicated that all the sialic acid residues were in the α-D configuration, and this was confirmed by the ^{13}C NMR spectrum of the native type Ia antigen. The characteristic chemical shift of the carboxylate (Na^+) carbon (1E) at 174.8 ppm is indicative of the sialic acid residues having the α-D configuration (29, 30). Removal of the sialic acid from the native polysaccharide ($[\alpha]_D = -2°$ in water) gave the degraded antigen, which had specific rotation and ^{13}C NMR spectrum identical to those of the HCl antigen and which also yielded the same oligosaccharide after the modified Smith degradation.

Methylation analysis of the native type Ia antigen established that all the sialic acid residues were present as single nonreducing end-groups, because in the methanolysis of the permethylated antigen only full methylated derivatives of sialic acid were detected (27). Hydrolysis of the methylated antigen and reduction and acetylation of the products yielded the partially methylated alditol acetates shown in Table 1. The difference between these partially methylated sugars and those yielded by the incomplete antigen clearly reflect the structural relationship between the native and incomplete antigen. In addition to fully methylated sialic acid residues, the native antigen also yielded a 2,4,6-tri-*O*-methylgalactopyranose residue, which replaced the 2,3,4,6-tetra-*O*-methylgalactopyranose derivative of the incomplete antigen. This is indicative of sialic acid residues (E) being linked to O-3 of the peripheral end-group galactopyranose residues (D) of the incomplete antigen. The fact that no 2,3,4,6-tetra-*O*-methylgalactopyranose was detected in the methylated type Ia native antigen is consistent only with its being composed of a repeating unit (Fig. 2) in which all the peripheral β-D-galactopyranose residues of the incomplete antigen are substituted.

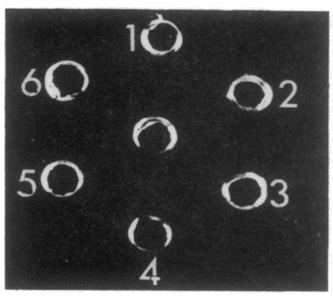

FIG. 3. Double diffusion in agar demonstrating the specificity of the Ia antigen (center well) reaction with serotype-specific Ia serum (well 4). There is no reaction with sera specific for type II (well 1), type Ic (well 2), type Ib (well 3), or type III (well 6).

Serological properties of the native type Ia antigen

The serological specificity of the type Ia native antigen was demonstrated in immunodiffusion tests using various group B streptococcal type-specific antisera (Fig. 3). The antigen was used at a concentration of 1 mg/ml and precipitated only with the type Ia-specific sera. Type Ia-specific serum failed to precipitate the structurally related fetuin.

DISCUSSION

The incomplete core polysaccharide antigens of group B *Streptococcus* types Ia (7), Ib (8), II (5), and III (6, 9, 10) are composed of the same sugars (galactose, glucose, and 2-acetamido-2-deoxyglucose), and the antigens of types Ia, Ib, and III have these sugars in identical molar ratios. Despite these similarities, the type Ia, II, and III incomplete polysaccharide antigens retain their serological specificity (1–4). In the case of the type Ia (*vide infra*) and type III (10) incomplete polysaccharide antigens, this is due to their differing structural arrangements. Both polysaccharides still retain two common structural features in the form of terminal β-D-galactopyranose residues and a β-D-GlcNAc*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p* trisaccharide unit. However they differ in that the terminal β-D-galactopyranose residues of the type Ia polysaccharide are linked to O-4 of the main chain β-D-galactopyranose unit instead of the 2-acetamido-2-deoxy-β-D-glucopyranose units as they are in the type III polysaccharide (10). In addition, the main chain trisaccharide unit common to both types is linked at O-4 of the 2-acetamido-2-deoxy-β-D-glucopyranose unit of the type Ia polysaccharide instead of at O-6 of the same unit in the type III polysaccharide (10). On the basis of inhibition studies (5, 11) it has been proposed that the type II incomplete antigen also contains terminal β-D-galactopyranose residues; together with the known serological specificity of the type Ia, II, and III antigens this observation indicates that this residue cannot itself be a determinant in the production of antibodies to the type Ia, II, and III organisms (1–4). Recently it has been demonstrated that the purified type Ib incomplete antigen crossreacts with type Ia and type Ic sera (8). Therefore the common structural features responsible for this crossreaction must reside wholly or partly in the structure of the type Ia incomplete polysaccharide antigen. It is also likely that this structural feature could be involved in the Iabc determinant (2, 8), although sialic acid residues may also play a role.

On the basis of degradative studies it has long been suspected that the native type Ia (12, 14), Ib (8), II (5, 11, 13), and III (15) antigens are all related to their respective incomplete core (HCl) antigens by the addition of peripheral sialic acid residues. Structural studies have confirmed the terminal position and the α-D configuration of all the sialic acid residues of both the type III (10) and type Ia (Fig. 2) native antigens. However, the serological specificity of the latter two antigens would necessitate that the sialic acid residues of neither antigen are serological determinants. One basic difference between the type Ia and type III native antigens is that the sialic acid residues of the former are linked to O-3 and the latter to O-6 (10) of their respective adjacent side-chain β-D-galactopyranose residues. Thus the terminal α-D-NeuAc*p*-(2→3)-β-D-Gal*p* unit of the type Ia antigen, like that of the equivalent α-D-NeuAc*p*-(2→6)-β-D-Gal*p* unit of the type III antigen is structurally related to animal and human glycoprotein. The former disaccharide exists as end group in both the multiple *O*- and *N*-tetrasaccharide units of fetuin (31) and the important human M and N blood group substances (32), whereas the latter disaccharide is found as end group in human (33) and rabbit (34) serotransferrin. This implies that, like the type III antigen (10), the specificity of the

native type Ia antigen must be based on a determinant larger than the side-chain α-D-NeuAc*p*-(2→3)-β-D-Gal*p* unit, thus involving main-chain constituents. Evidence for this can be obtained from immunodiffusion studies, in which the streptococcal type Ia antiserum formed a strong precipitin line with its homologous native polysaccharide but did not react with fetuin.

It has been demonstrated that, like the type III native antigen (10), type Ia native antigen extracted at pH 7.0 from organisms grown under pH control (pH 7.0) has all of its side-chain β-D-galactopyranose residues masked by peripheral sialic acid groups. This is in contrast to the findings of Kane and Karakawa (7), who provided evidence for the presence of both terminal galactose and sialic acid residues in their preparation of the type Ia native antigen. Our results would indicate that they obtained a partially degraded antigen despite the fact that they had maintained pH control (pH 7.0) during the extraction procedures. Thus, although they failed to record any pH data from the growth conditions that they employed (7, 35), it seems reasonable to propose that the acidity of their growth medium could be implicated in the removal of some of the labile sialic acid residues from the cell-associated native polysaccharide. Certainly this lack of pH control during the growth of the type III organisms subsequently used to raise antisera in rabbits (Lancefield procedures) has been shown to be a major factor in the production of antibodies to determinants terminating in β-D-galactopyranose residues (10). Using procedures identical to those of Kane and Karakawa (7), we also obtained antisera to the type Ia organisms that contained antibodies to determinants terminating in β-D-galactopyranose residues.

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1. Lancefield, R. C. (1933) *J. Exp. Med.* **57,** 571–582.
2. Lancefield, R. C. (1934) *J. Exp. Med.* **59,** 441–458.
3. Lancefield, R. C. (1938) *J. Exp. Med.* **67,** 25–40.
4. Wilkinson, H. W. & Eagon, R. (1971) *Infect. Immun.* **4,** 596–604.
5. Lancefield, R. C. & Friemer, E. H. (1966) *J. Hyg.* **64,** 191–203.
6. Russell, H. & Norcross, N. L. (1972) *J. Immunol.* **109,** 90–96.
7. Kane, J. A. & Karakawa, W. W. (1978) *Infect. Immun.* **19,** 983–991.
8. Tai, J. Y., Gotschlich, E. C. & Lancefield, R. C. (1979) *J. Exp. Med.* **149,** 58–66.
9. Kasper, D. L., Baker, C. J., Baltimore, R. S., Crabb, J. H., Schiffman, G. & Jennings, H. J. (1979) *J. Exp. Med.* **149,** 327–339.
10. Jennings, H. J., Rosell, K.-G. & Kasper, D. L. (1980) *Can. J. Biochem.* **58,** 112–120.
11. Freimer, E. H. (1967) *J. Exp. Med.* **125,** 381–392.
12. Wilkinson, H. W. (1975) *Infect. Immun.* **11,** 845–852.
13. Baker, C. J. & Kasper, D. L. (1976) *Infect. Immun.* **13,** 284–288.
14. Kane, J. A. & Karakawa, W. W. (1977) *J. Immunol.* **118,** 2155–2160.
15. Baker, C. J., Kasper, D. L. & Davis, C. E. (1976) *J. Exp. Med.* **143,** 258–270.
16. Kasper, D. L., Goroff, D. K. & Baker, C. J. (1978) *J. Immunol.* **121,** 1096–1105.
17. McCarty, M. & Lancefield, R. C. (1955) *J. Exp. Med.* **102,** 11–35.
18. Kabat, E. A. & Mayer, M. M. (1961) *Experimental Immunochemistry* (Thomas, Springfield, IL), pp. 85–88.
19. Dmitriev, B. A., Backinowsky, L. V., Lvov, V. L., Kotchetkov, N. K. & Hoffman, I. L. (1975) *Eur. J. Biochem.* **50,** 539–547.
20. Sawardeker, J. S., Sloneker, J. H. & Jearls, A. R. (1965) *Anal. Chem.* **37,** 1602–1604.
21. Leontein, K., Lindberg, B. & Lönngren, J. (1978) *Carbohydr. Res.* **62,** 359–362.
22. Warren, L. (1963) *Methods Enzmol.* **6,** 463–465.
23. Kuhn, R., Lutz, P. & MacDonald, D. L. (1966) *Chem. Ber.* **99,** 611–617.
24. Hakomori, S. (1964) *J. Biochem.* **55,** 205–208.
25. Lindberg, B. (1972) *Methods Enzymol.* **28,** 178–195.
26. Stellner, K., Saito, H. & Hakamori, S. (1973) *Arch. Biochem. Biophys.* **155,** 464–472.
27. Bhattacharjee, A. K. & Jennings, H. J. (1976) *Carbohydr. Res.* **51,** 253–261.
28. Lindberg, B., Lonngren, J. & Powell, D. A. (1977) *Carbohydr. Res.* **58,** 177–186.
29. Jennings, H. J. & Smith, I. C. P. (1978) *Methods Enzymol.* **50,** 39–50.
30. Jennings, H. J. & Bhattacharjee, A. K. (1977) *Carbohydr. Res.* **55,** 105–112.
31. Spiro, R. G. & Bhoyroo, V. D. (1974) *J. Biol. Chem.* **249,** 5704–5717.
32. Sadler, J. E., Paulson, J. C. & Hill, R. L. (1979) *J. Biol. Chem.* **254,** 2112–2119.
33. Spik, G., Bayard, B., Fournet, B., Strecker, G., Bougelet, S. & Montreuil, J. (1975) *FEBS Lett.* **50,** 269–299.
34. Leger, D., Tordera, V., Spik, G., Dorland, L., Haverkamp, J. & Vliegenthart, J. F. G. (1978) *FEBS Lett.* **93,** 255–260.
35. Pazur, J. H., Anderson, J. S. & Karakawa, W. W. (1971) *J. Biol. Chem.* **246,** 1793–1798.