Pure & Appl. Chem., Vol. 56, No. 7, pp. 893-905, 1984.
Printed in Great Britain.

0033-4545/84 $3.00+0.00
Pergamon Press Ltd.

STRUCTURE, CONFORMATION AND IMMUNOLOGY OF SIALIC ACID-CONTAINING POLYSACCHARIDES OF HUMAN PATHOGENIC BACTERIA

Harold J. Jennings, Ewa Katzenellenbogen, Czeslaw Lugowski, Francis Michon, René Roy and Dennis L. Kasper*

Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada, K1A OR6 and *Channing Laboratory, Harvard Medical School, Boston, MA 02115, U.S.A.

Abstract - Capsular polysaccharides of types Ia, Ib, II and III Group B *Streptococcus* and groups B and C *Neisseria meningitidis* contain terminal sialic acid in different molecular environments. Experimentation has identified sialic acid as an important factor in the virulence of these organisms and in the human antibody response to their capsular polysaccharide antigens. Although terminal sialic acid is not normally immunogenic it controls the determinants which are responsible for the production of protective antibodies.

Using immunological and NMR spectroscopic techniques on the native and specifically modified polysaccharides, a number of these sialic acid-controlled determinants have been identified and located. These determinants are only formed in structures which can accommodate long-range interactions between sialic acid and other remote glycosyl residues. The carboxylate group of sialic acid is essential for these interactions to occur.

INTRODUCTION

Because of their surface location on bacteria, capsular polysaccharides are at the interface of many host-bacteria interactions (1,2). They constitute the major antigens of many pathogenic bacteria and in consequence have been utilized in a number of human vaccines against disease caused by these bacteria (1,3). Capsular polysaccharides are important virulence factors in bacterial disease, and although no single structural feature has been identified to account for the virulence of certain bacteria, it is more than circumstantial that all bacteria with capsular polysaccharides having terminal sialic acid are effective human pathogens (1,4). Experiments using whole bacteria having this type of capsule in a desialylated and chemically modified (5) form have demonstrated that sialic acid is able to inhibit the activation of the alternative pathway of complement, and it is not unreasonable to suppose that this structural feature was an evolutionary acquisition by bacteria to increase their potential to evade the human immune system (6). The capsular polysaccharides of types Ia, Ib, II and III Group B *Streptococcus* and groups B and C *Neisseria meningitidis* all have terminal sialic acid and all are human pathogens (1,4). Therefore they are currently in use or are being considered for use as human vaccines (1,3,4). The human immune response to the type III group B streptococcal polysaccharide has revealed another important function of terminal sialic acid, in that it is essential to the formation of the determinant responsible for the production of human protective antibodies, despite the fact that it is not immunogenic (1,4,7). The hypothesis proposed to explain the above phenomenon is that sialic acid is able to exert conformational control over determinants remote from itself, by through-space interactions with other glycosyl residues in the polysaccharide (7,8). Recently a similar proposal was also made to explain unusual long-range chemical shifts in the ^{13}C-NMR spectra of some sialooligosaccharides obtained from human urine (9).

The aforementioned polysaccharides have been structurally defined (10-12) and serve as useful models to study the conformational function of sialic acid when it is situated in a number of different molecular environments. Immunological and NMR spectroscopic studies on these polysaccharides were

aimed at confirming the above hypothesis and attempting to establish the factors involved in this function of sialic acid.

This phenomenon could be important as it provides a plausible explanation for the fact that despite its ubiquity in nature, terminal sialic acid is involved in many diverse and highly specific recognition processes (13). Certainly this phenomenon probably occurs frequently in nature, as there is NMR spectroscopic evidence indicative of the possibility of conformational control by sialic acid in glycoprotein (9) and gangliosides (14,15) and immunological evidence which is consistent with its occurrence in blood group substances (16).

RESULTS AND DISCUSSION

Polysaccharide structures

The structures of the specific capsular polysaccharide antigens of groups B and C Neisseria meningitidis and types Ia, Ib, II and III group B Streptococcus are shown in Table 1 and all contain sialic acid. The groups

TABLE 1. Structures of capsular polysaccharides

Type	Structure
	Group B Streptococcus
Ia	→4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→ 3 ↑ 1 β-D-Glc*p*NAc 4 ↑ 1 α-Neu*p*NAc-(2→3)-β-D-Gal*p*
Ib	→4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→ 3 ↑ 1 β-D-Glc*p*NAc 3 ↑ 1 α-Neu*p*NAc-(2→3)-β-D-Gal*p*
II	→4)β-D-Glc*p*NAc-(1→3)β-D-Gal*p*(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-β-D-Gal*p*(1→ 6 3 ↑ ↑ 1 2 β-D-Gal*p* α-Neu*p*NAc
III	→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→ 4 ↑ 1 α-Neu*p*NAc-(2→6)-β-D-Gal*p*
	Neisseria meningitidis
Group	Structure
B	→8)-α-Neu*p*NAc-(2→8)-α-Neu*p*NAc→
C	→9)-α-Neu*p*NAc-(2→9)-α-Neu*p*NAc→

B and C meningococcal polysaccharides are both homopolymers of sialic acid, different strains of group C organisms producing the same homopolymer with and without O-acetyl substituents (17,18). In this paper for the sake of simplicity the latter homopolymer will be referred to as the group C polysaccharide. Because of the uniqueness of polysaccharides containing sialic acid, and because of their extreme fragility, the structure of these polysaccharides was more readily deduced using ^{13}C-NMR spectroscopy than by using more conventional techniques. The ^{13}C-NMR spectra of the groups B and C polysaccharides, the former shown in Fig. 1, both exhibited only eleven

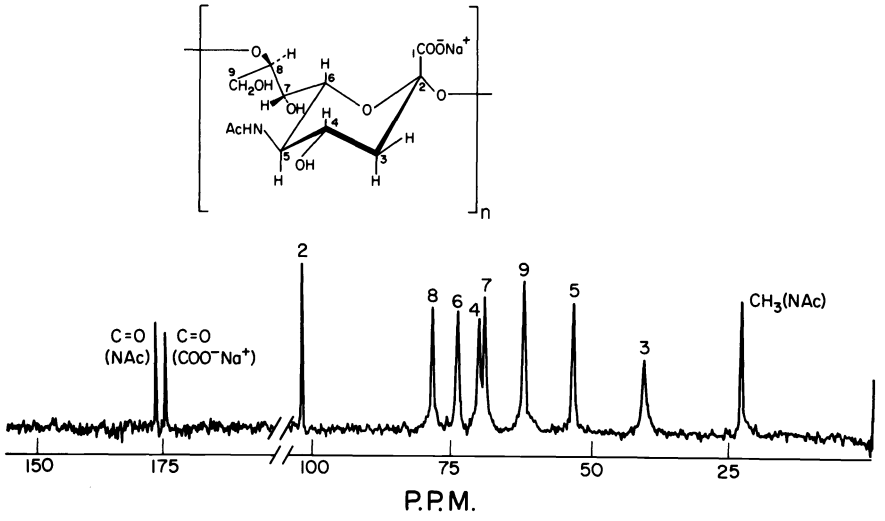


Fig. 1 100 MHz-NMR spectrum of the group B meningococcal polysaccharide.

signals, each signal representing a different carbon atom of their sialic acid repeating units. The simplicity of the spectra indicated that both the groups B and C polysaccharides were linear homopolymers of sialic acid each containing only one type of linkage. By comparing the above spectra with those of monomeric sialic acid methyl glycosides it was possible to deduce from the chemical shifts of the signals assigned to C1, C4 and C6 that the sialic acid residues in both polysaccharides were in the α-configuration. That the sialic acid residues of the group B polysaccharide were linked α-(2→8) and those of the C polysaccharide α-(2→9) was deduced by characteristic chemical shift displacements in carbons associated with, or vicinal to, the linkages (17).

The native types Ia, Ib and III group B streptococcal antigens contain D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose and sialic acid in the molar ratios of 2:1:1:1, and constitute a group of isomeric heteropolysaccharides (10,11) whereas the type II native antigen has the same component sugars but in a different molar ratio (3:2:1:1 respectively) (12). These structures were elucidated in large part by comparison of the methylation analyses of the native antigens with those of the corresponding specifically modified antigens produced by the sequential removal of component sugars from the branches of the native polysaccharides. These modified antigens also proved to be useful in immunological specificity studies. All the polysaccharides contain terminal sialic acid residues and a common β-D-GlcpNAc-(1→3)-β-D-Galp-(1→4)-β-D-Glcp trisaccharide which forms the repeating unit of the backbone of the type III polysaccharide. In the types Ia, Ib and II antigens the terminal sialic acid is α-(2→3)-linked to β-D-galactopyranosyl residues, while in the type III antigen it is α-(2→6)-linked to the same residue .

Biological and immunological significance

Structurally the meningococcal groups B and C and the group B streptococcal antigens are of interest because of their relationship to each other and to other biologically important molecules. These polysaccharides are representative structures in which sialic acid is situated in a variety of different molecular environments and they can be conveniently classified as linear polymers having -

1. disaccharide branches terminating in sialic acid (type III polysaccharide).
2. trisaccharide branches terminating in sialic acid (types Ia and Ib polysaccharides).
3. monomeric sialic acid branches (type II polysaccharide).
4. terminal and internal sialic acid (groups B and C polysaccharides).

These polysaccharides are of interest because they are immunogenic, and largely immunospecific, despite the fact that they all contain terminal sialic acid and other more extensive structural similarities. This is because terminal sialic acid is not normally immunogenic due to its ubiquitous occurrence in human and animal tissue (6). This reasoning also applies to larger sialooligosaccharides in the polysaccharides. For example, the terminal α-NeupAc-(2→3)-β-D-Galp unit of the types Ia and Ib polysaccharides has been identified as end-group in both the multiple O- and N-tetrasaccharide units of fetuin (19) and the important human M and N blood group substances (20), while the equivalent α-(2→6)-linked disaccharide of the type III polysaccharide exists as end-group in human serotransferrin (21). Oligosaccharides consisting of linear α-(2→8)-linked sialic acid, a structural feature of the group B polysaccharide, have also been identified in the gangliosides of human and animal brain tissue (22-25).

Despite the fact that sialic acid is not normally immunologically expressed it still remains immunodominant in a non-classical sense (26). Thus we have a unique situation, where although sialic acid may not even be a part of a determinant, it is still able to exert conformational control over determinants involving more remote domains of the polysaccharide. This fact has important implications in the formulation of human polysaccharide vaccines. For example, in the possible use of polysaccharide vaccines against meningitis caused by group B streptococcal organisms. Although it is possible for humans to produce numerous antibody populations with different specificities to the type III antigen, it is only antibody made to the sialic acid-controlled determinant that is protective (4).

Using serological techniques a number of conformational determinants have been identified in the above polysaccharides, and by correlating serological and NMR data on the native and specifically modified native polysaccharides we have located and attempted to define some of these conformational determinants with greater precision.

Type III polysaccharide determinants

Immunization of rabbits with type III group B streptococcal organisms induces two distinct populations of antibodies with a specificity for determinants on the type III native capsular polysaccharide (8). That the specificity of the determinant corresponding to the major population of

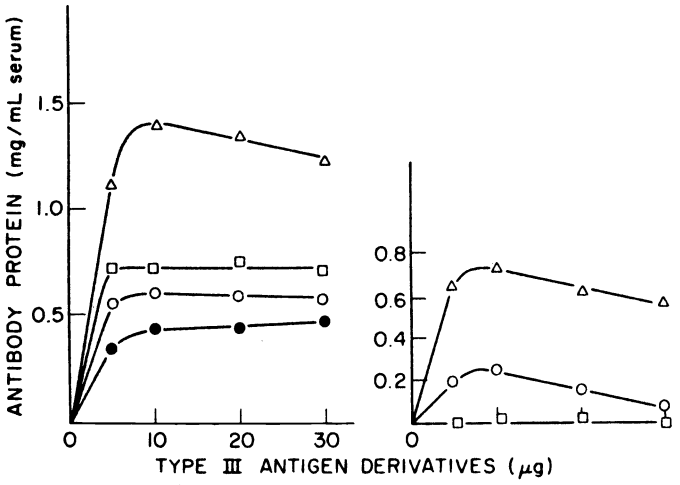


Fig. 2 Quantitative precipitin analysis of the native (Δ), reduced native (□), core (o) and backbone (•) type III antigens with type III Group B Streptococcal antiserum (left), and with the same antiserum previously absorbed with the type III reduced native antigen (right).

antibodies is dependent on the presence of sialic acid on the type III antigen was demonstrated by immunodiffusion and quantitative precipitin experiments, with this antiserum, using the native (structure shown in Table 1), carboxyl-reduced native, desialylated (core), and degalactosylated core (backbone) type III antigens (8). The quantitative precipitin experiments are shown in Fig. 2 and demonstrate that only the native type III antigen is the complete antigen, the chemically modified antigens being capable of only partial precipitation and absorption of the total antibody. On the basis of these serological experiments the total antibody can be conveniently divided into two populations, one with and one without dependence on the presence of sialic acid on the type III antigen. From these experiments it can also be deduced that the backbone is the site of the determinant responsible for the production of the latter antibody population. It is of interest to note that while sialic acid is involved in the formation of the determinant responsible for the former antibody population, because of the immunological specificity exhibited by the group B streptococcal polysaccharides and the fact that they all have terminal sialic acid residues, it cannot be immunodominant in the classical sense (26). One possible explanation for this phenomenon could be that specificity is achieved via a much larger determinant of which sialic acid is only a small part. However, this possibility was rejected when in inhibition studies both serotransferrin and the type III core antigen, having all the additive and overlapping features of the native type III antigen, proved to be poor inhibitors of the homologous serological reaction (8).

Therefore the conformational dependence of this determinant was postulated in which the terminal sialic acid, and in particular because of the serological equivalence of the reduced native and core type III antigens (Fig. 2), the carboxylate group of these residues, must play an important part. Confirmatory evidence for the occurrence of such a conformational change in the determinant, due to the loss or reduction of these sialic acid residues, can be obtained by comparing the chemical shifts of the anomeric and linkage carbons in the ^{13}C-NMR spectra of the native, reduced native and core type III antigens (Fig. 3). Chemical shift displacements involving these particular carbons can be indicative of inter-glycose conformational changes (27,28). In the spectrum of the native type III antigen (Fig. 3) one anomeric signal at 103.6 p.p.m. and one of the three linkage signals at

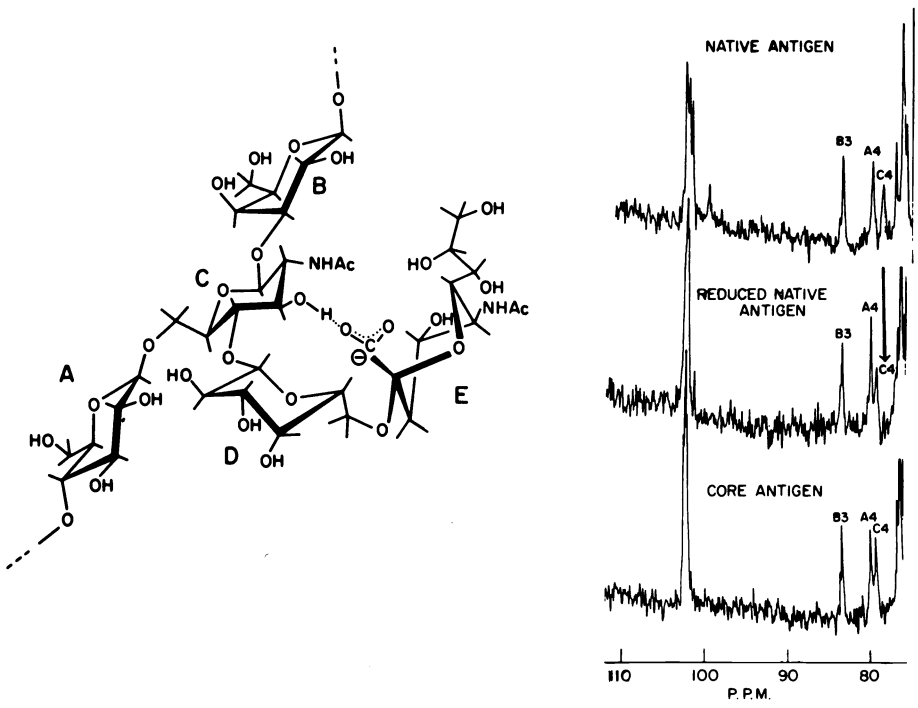


Fig. 3 Proposed conformation of the repeating unit of the type III polysaccharide (left), and a spectral window on the 90 MHz ^{13}C-NMR spectra of type III antigens (right).

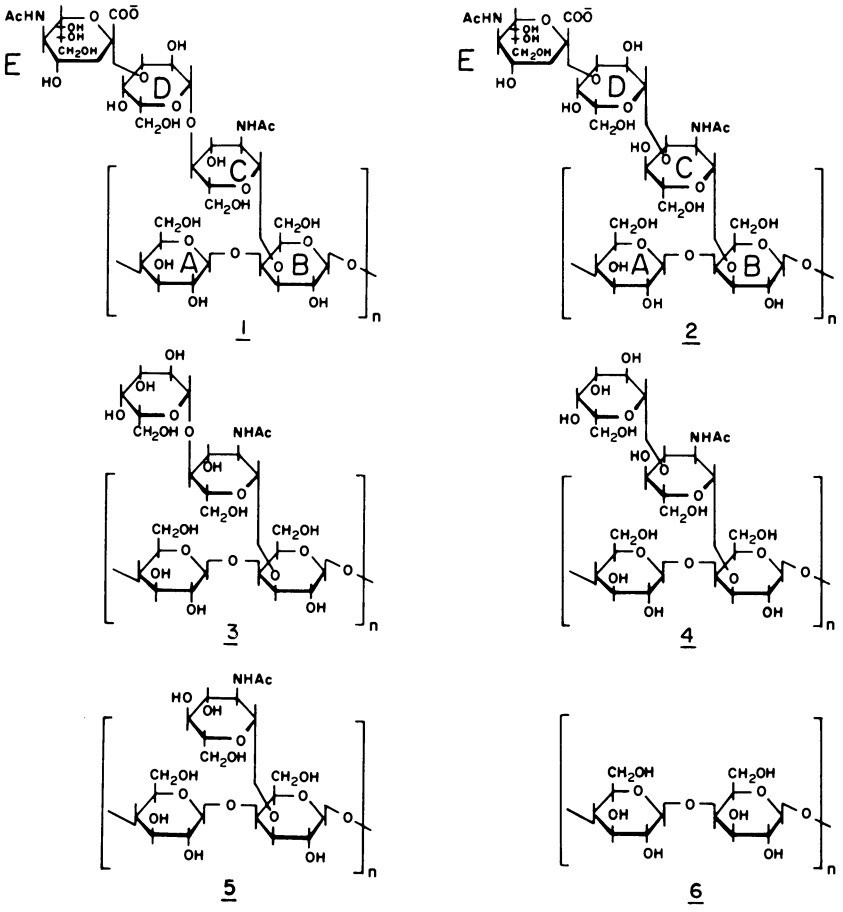
78.3 p.p.m. underwent significant displacements on carboxyl-reduction or removal of sialic acid from the native type III antigen. Using the backbone antigen as a model in ^{13}C-NMR spectroscopic studies, it was possible to assign the displaced linkage signal to C-4 of the 2-acetamido-2-deoxy-β-D-glucopyranosyl residues (C) in the repeating unit of the native type III polysaccharide (Fig. 3). This evidence indicated a conformational change involving the branches of the native type III antigen, rather than more extensive conformational changes in its backbone, and is consistent with the serological evidence in which the antigenicity of the backbone determinant was found to be independent of the presence of terminal sialic acid residues. The proposed conformation of the native type III antigen is shown in Fig. 3 and its formation probably involves interactions between terminal sialic acid (E) and the penultimate 2-acetamido-2-deoxy-β-D-glucopyranosyl residues (C) of the backbone. Models (CPK) indicate that it is possible for the sialic acid residues of the repeating unit shown in Fig. 2 to be situated in close proximity to these latter residues (C), and all the evidence is still consistent with the possibility that hydrogen bonding could be a factor in the inter-glycose interactions which stabilize this conformation. No evidence for such a conformational change could be obtained using high resolution ^{1}H-NMR, because there were no significant chemical shift displacements in the anomeric protons of native type III polysaccharide when it was desialylated or carboxyl-reduced (29).

A recent analysis (9) of the ^{13}C-NMR chemical shift data of the identical sialotrisaccharide involved in the formation of the conformational determinant (residues E, D and C) also confirms the aforementioned postulate. On removal of terminal sialic acid from the above trisaccharide, equivalent large chemical shift displacements were observed for carbons D1 and C4 which were rationalized on the basis of conformational changes instigated by strong interactions between sialic acid (E) and residue C. Conformational changes also occurred in the equivalent trisaccharide where residue C was β-D-glucopyranose. However the formation of such a conformation involving the above trisaccharide cannot solely be responsible for the specificity of the native type III determinant, because the identical trisaccharide failed to inhibit the homologous serological reaction of the native type III antigen. Obviously this serological specificity must also depend on backbone residues, although the participation of these residues must be limited due to the fact that the core antigen is also a poor inhibitor of the above serological reaction (8). The participation of backbone residues in the conformational determinant has been confirmed in recent preliminary serological studies (30). In these studies it has been demonstrated that the pentasaccharide repeating unit, having the same sequence as depicted in Fig. 3, does have inhibitory properties in the homologous serological reaction of the native type III polysaccharide.

Types Ia and Ib polysaccharide determinants

The types Ia (1) and Ib (2) polysaccharide antigens of group B Streptococcus are isomers, having trisaccharide branches terminating in sialic acid. The structural elucidation of these polysaccharides was achieved largely by methylation analyses of a series of specifically modified antigens in which terminal glycoses were sequentially removed from their trisaccharide branches, to yield their desialylated (3 and 4), degalactosylated (5), and deglucosaminylated backbone (6) antigens. These modified antigens were also employed in both immunological and NMR spectroscopic studies aimed at identifying and locating possible conformational determinants. The types Ia and Ib polysaccharides have extensive regions of structural similarity. Both 1 and 2 have identical degalactosylated antigens (5) and common terminal α-NeupAc-(2→3)-β-D-Galp units. They differ only in the linkages between their branch β-D-galactopyranosyl (D) and 2-acetamido-2-deoxy-β-D-glucopyranosyl (C) residues. In 1 these residues are (1→4)-linked and in 2 they are (1→3)-linked. This small structural difference between the two polysaccharides has a profound effect on their individual immunological properties.

Like the type III group B streptococcal organisms, those of type Ia produce two distinct populations of antibodies in rabbits, one of these populations being dependent on the presence of terminal sialic acid in the type Ia antigen. This was demonstrated in quantitative precipitation experiments using the above antiserum (29) in which the native Ia antigen was still able to precipitate further significant quantities of antibody following absorption of the antiserum with 3 and carboxyl-reduced 1. Although the types Ia and Ib polysaccharides are weakly cross-reactive, which is



incidentally due to their common degalactosylated antigen (29), their immunological specificity, when compared to the other group B streptococcal polysaccharides, would mitigate against sialic acid being a part of the determinant responsible for this latter antibody. Therefore as in the case of the native type III antigen, we can postulate the function of sialic acid as being one of conformational control over determinants in the native type Ia polysaccharide.

Unfortunately we have been unable to locate this determinant from either ^{1}H- or ^{13}C-NMR chemical shift data (29). Although some displacement of anomeric signals, not associated with sialic acid, were observed in comparing the ^{13}C-NMR spectra of the native and core Ia antigen, no equivalent displacement in the linkage signals was observed. Both 1 and 3 exhibit two linkage signals at 82.9 and 79.7 p.p.m. in the intensity ratio of 1:2. On the basis of previous assignments the former signal could be assigned to the carbon atom at B3 and the latter to those at C4 and A4. The signals of the carbon atoms at B4 and D3 were unobservable because they were hidden in a higher field complex group of overlapping signals. However it is highly unlikely that torsion angle changes at B4 could alone explain the formation of the serologically-detected conformational determinant because 5 is still able to precipitate some of the antibody made to the native Ia polysaccharide. The fact that 6 is unable to precipitate any of the above antibody indicates that the branches of the native Ia antigen must be involved in all the determinants responsible for the polysaccharide specific antibodies. Because the carboxyl-reduced 1 is serologically equivalent to 3 one can rationalize all the above evidence if one proposes that, as in the case of the native type III antigen, the sialic acid residues of the native Ia polysaccharide interact, by virtue of their proximity, with another as yet unidentified part of the molecule. However, it is unlikely that, as in the case of the native type III polysaccharide, this interaction could occur with the penultimate 2-acetamido-2-deoxy-β-D-glucopyranosyl residue (C).

This is because models (CPK) of the terminal trisaccharide (α-NeupAc-(2→3)-β-D-Galp-(1→4)-β-D-GlcpNAc) indicate that these two residues cannot be placed in close proximity to each other, and more convincingly because of the fact that the type Ib polysaccharide (2), which has an identical terminal trisaccharide, does not form such a conformational determinant (see later). Therefore it is necessary to propose that this interaction occurs with even more remote parts of the molecule, e.g. the backbone or another branch.

The above hypothesis is attractive because it provides an explanation for the apparently conflicting serological and ^{13}C-NMR spectroscopic results. Such a long-range interaction would minimize distortion of the torsion angles of linkages in the branches of the native Ia antigen because this distortion could be absorbed by more than one linkage. Thus the torsion angle changes in each individual linkage might not be large enough to result in detectable chemical shift displacements in the signals of the linkage carbons. Also from a serological point of view these conformational changes may be small enough that they would not necessarily impair the ability of the antibodies, made exclusively to the branches of the native Ia antigen, from still recognizing the equivalent domains on the branches of the core antigen. The serological specificity of the sialic acid determinant must therefore be dependent on its binary nature being situated across two domains of the native Ia polysaccharide, adjacent in space, at least one of these domains being situated on the branches of the native Ia polysaccharide. Nulification of the long-range interaction involving sialic acid, by its carboxyl-reduction or removal, would then result in sufficient separation of these domains to cause lack of recognition by these antibodies.

Contrary to type Ia group B streptococcal organisms, when injected in rabbits, those of type Ib produce no sialic acid-dependent antibody to the native type Ib polysaccharide. This was demonstrated in serological studies (29), when 2 and 4 were able to precipitate the same amount of the above homologous antiserum previously absorbed with 5. Thus the type Ib polysaccharide constitutes a unique structure in which sialic acid is not immunodominant in either a direct or indirect sense. By virtue of its spatial location, terminal sialic acid is incapable of interacting with other branch components or other remote regions of the type Ib polysaccharide, thus ensuring the serological equivalence of the native (2) and core (4) Ib polysaccharides. Because 1 and 2 only differ in the position of linkage between residues C and D, this must be the critical structural feature which determines whether interaction occurs or not.

Because of a determinant situated on 5, the types Ia and Ib polysaccharides do cross-react to a certain extent (29,30). However this cross-reaction is minimal despite the fact that the degalactosylated antigen is very strongly expressed in the type Ib antiserum. Obviously these determinants are not readily available on the type Ia native or core antigens which could be due to differences in shielding of the degalactosylated antigen (5) by branch component sugars.

Type II polysaccharide determinant

One of the unique features of the type II polysaccharide, as opposed to the other group B streptococcal polysaccharides, is that it has sialic acid attached directly to its backbone. Two distinct populations of antibodies have been defined in antisera raised in mice and rabbits immunized with whole group B streptococcal organisms (32), and it has been demonstrated that one population of antibodies is dependent on the presence of sialic acid. This is exemplified by the ability of the native type II polysaccharide to precipitate the above antiserum even following absorption of this antiserum with the desialylated (core) antigen (Fig. 4). The type II polysaccharide also differs from the other group B streptococcal polysaccharides in that it has terminal β-D-galactopyranosyl residues. This structural feature was proposed earlier on the basis of inhibition studies (33,34), in which D-galactose was shown to have strong inhibitory properties. This has been corroborated in more recent serological studies in which it was demonstrated (31) that D-galactose was capable of inhibiting populations of antibodies in a type II antiserum precipitating both the native and core polysaccharides.

Although the type II native polysaccharide is slightly cross-reactive with that of the type Ia, presumably due to the fact that they share a common sialopentasaccharide, sialic acid is not immunologically expressed, because

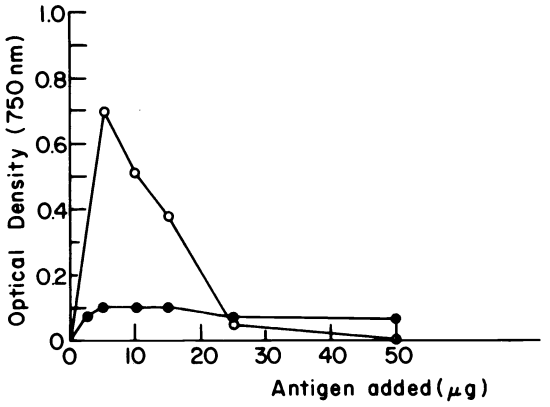
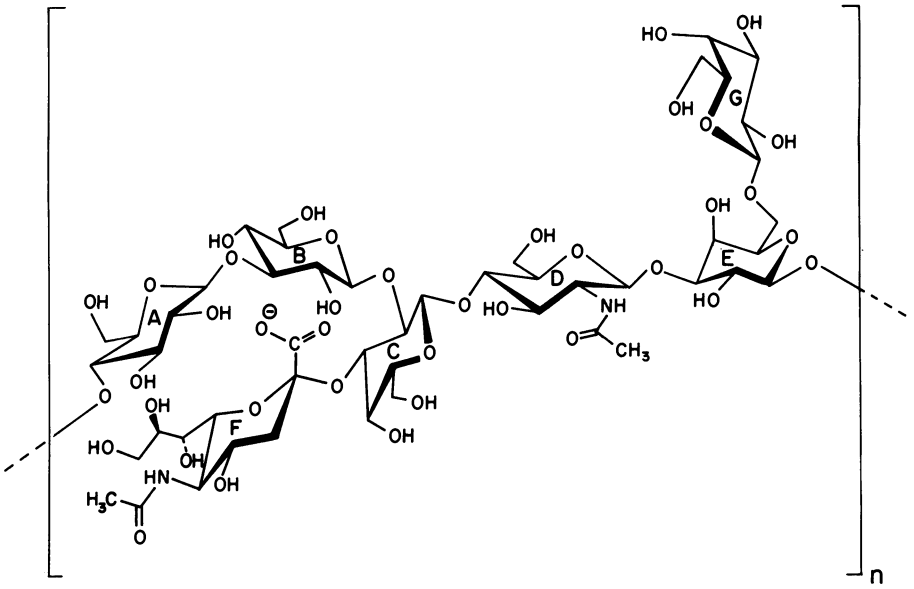


Fig. 4 Quantitative precipitin analysis of the native type II antigen (o) with the type II Group B Streptococcal antiserum previously absorbed with the type II core antigen, and of the type II core antigen (•) with the same antiserum previously absorbed with the type II native antigen.

no such cross-reaction occurs with the isomeric type Ib polysaccharide (31). However, because sialic acid is a necessary adjunct to the determinant responsible for the major population of the antibodies to the type II native antigen, one must again suspect the presence of a sialic acid-controlled conformational determinant.

Using a combination of serological and NMR spectroscopic data it was possible to locate the critical structural feature involved in this determinant (35). The structure of the repeating unit of the type II antigen is shown in 7, and assuming that it is analogous to the types Ia and III group B streptococcal polysaccharides, the conformational determinant is probably generated by interactions between sialic acid and other domains of the polysaccharide. One can deduce that this interaction must be localized to regions of the backbone of 7 in the vicinity of the sialic acid residues because these latter residues are only present as single branched residues. However, an examination of 7 indicates that theoretically two such



7

interactions are possible, one to the left, and one to the right of the terminal sialic acid residue. All the available evidence indicates that this interaction occurs to the left of the sialic acid residue in 7. A short-range interaction to the right of the sialic acid residue would not be consistent with the lack of flexibility of α-NeupNAc-(2→3)-β-D-Galp-(1→4)-β-D-GlcpNAc previously demonstrated in the type Ia native polysaccharide which is also consistent with the NMR chemical shift data below.

In comparing the ^{13}C-NMR spectra of 7, carboxyl-reduced 7 and desialylated 7, displacements in the anomeric signals and one signal in the linkage region of the latter two spectra were observed. It was not possible to assign the displaced anomeric signals but the displaced linkage signal could be assigned, using previous assignments made on structures similar to 7. The ^{13}C-NMR spectrum of 7 exhibits four linkage signals at 84.7 (B3), 83.3 (E3) and 80.3 (A4 and D4) p.p.m., and only the signal at 84.7 p.p.m. was significantly displaced to 85.3 p.p.m. by both carboxyl-reduction and removal of the sialic acid residues. The above evidence is consistent with sialic acid, acting exclusively through its carboxylate group, creating changes in the torsion angles of the bonds between residues A and B of 7. This would require proposing interactions between sialic acid and residue A to form a four-residue ring system. Space-filling models (CPK) indicate that such an interaction is possible and the evidence is consistent with the fact that hydrogen bonding between the carboxylate group and the 2 OH of residue A could be one of the forces in this interaction, although obviously additional forces could also be involved.

The ^{1}H-NMR studies on 7 and carboxyl-reduced 7 (35) fully confirm the aforementioned hypothesis. The chemical shifts of the anomeric protons of the 7 and selectively modified 7 are shown in Table 2.

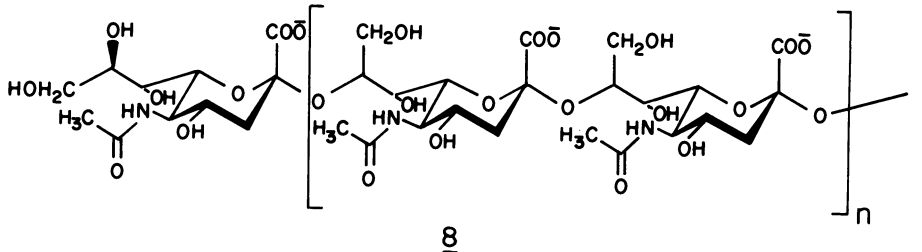
TABLE 2. Chemical shifts of the anomeric protons of the native (7) and selectively modified native type III polysaccharide

Residue	Polysaccharide			
	Native	Carboxyl-reduced native	Core	Backbone
A	4.84	4.74	4.75	4.78
B	4.80	4.73	4.80	4.72
C	4.72	4.73	4.74	4.72
D	4.59	4.59	4.56	4.54
E	4.45	4.43	4.43	4.43
F	4.45	4.42	4.43	-

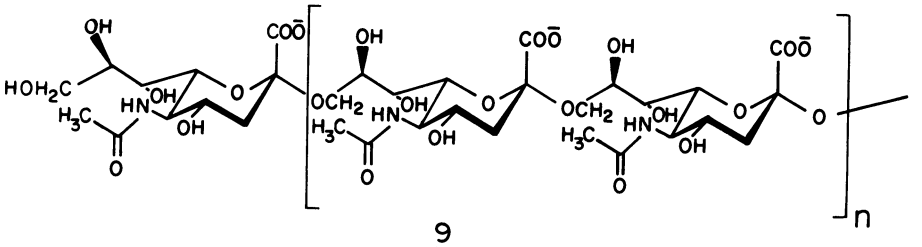
The chemical shifts were assigned using accumulated data obtained from a number of sources on a number of related model structures. Reduction of the carboxylate group of sialic acid resulted in significant displacements in two of the anomeric signals (4.84 and 4.80 p.p.m.) in the ^{1}H-NMR spectrum of the native type II antigen. These signals were assigned with some confidence to the anomeric protons of residues A and B respectively. Thus the carboxylate group is responsible for the deshielding effect on both anomeric protons, and for this effect to occur both protons must be in close proximity to the carboxylate group. This result not only confirms the existence of the proposed four-residue ring interaction but uniquely defines with some precision the geometry of the resultant conformational determinant. It is of interest to note that among all the group B streptococcal polysaccharides only the sialic acid residues of 7 are neuraminidase-resistant (12), and that this also applies to the structurally related GM1 ganglioside (II NeuAc-GgOSe$_4$Cer) (36).

Groups B and C polysaccharide determinants

The groups B and C polysaccharides of Neisseria meningitidis are homopolymers of sialic acid (17,18), the sialic acid residues being linked α-(2→8) in the former (8) and α-(2→9) in the latter (9). Because of this linkage difference the immunological properties of the polysaccharides differ profoundly. While the C polysaccharide is highly immunogenic, and is currently used as a constituent of a vaccine against meningococcal meningitis in humans (1,3), the B polysaccharide is only poorly immunogenic



8



9

(37) even when conjugated to a carrier protein (38). It is interesting to note that even in the Escherichia coli K92 polysaccharide, which contains alternating sequences of α-(2→8)- and α-(2→9)-linked sialic acid, only antibodies with a specificity for the latter linkage could be detected (39).

Whole group B organisms are able to produce low levels of B polysaccharide-specific antibodies in animals and humans, however, these antibodies are exclusively IgM and are of very low affinity (40). Possible reasons for the poor immunogenicity of the B polysaccharide include degradation of the polysaccharide by tissue neuraminidase, intramolecular esterification (41), and tolerance due to cross-reactive tissue components. None of the available evidence is supportive of either of the former two mechanisms and the identification of structures similar to 8 (n = 1 to 7) in the gangliosides of human and animal brain tissue (22-25) has lent credence to the latter argument. The sialoglycopeptides from human and rat brain bind to B polysaccharide-specific horse antibodies and inhibit the homologous serological reaction of these antibodies (25).

Although the B and C polysaccharides differ in most of their immunological properties they do have one property in common with each other and with the group B streptococcal polysaccharides, in that their terminal sialic acid residues are non-immunogenic (42). This was demonstrated for both polysaccharides when the methyl α-glycoside of sialic acid failed to inhibit the homologous serological reaction of either polysaccharide (42). However, unlike the Ia, II and III group B streptococcal polysaccharides the terminal sialic residues of 8 and 9 do not have individual functions in controlling the conformation of determinants.

To locate and define these determinants, the ability of a series of sialooligosaccharides to inhibit the homologous serological reaction of both polysaccharides was determined. The oligosaccharides were obtained by the partial hydrolyses of the polysaccharides (42). Except for the non-immunogenicity of its terminal sialic acid, the group C polysaccharide (9) behaved serologically like any other linear polysaccharide (42). The disaccharide unit of 9 was the minimum unit capable of inhibiting the homologous serological reaction and the ability of larger oligosaccharides to inhibit the reaction increased to a maximum at n = 2 or 3.

In contrast the B polysaccharide (8) exhibited very different properties in that no significant inhibition of its homologous serological reaction was obtained using the equivalent oligosaccharides 8 (n = 4 or 5) obtained from the B polysaccharide. This phenomenon has been observed in another laboratory (43) and is currently under investigation. Two possible reasons for the failure of the above oligosaccharides to inhibit are that either the minimum sized-determinant involved is large or that the determinant is

conformational in nature. This could be due to the presence of secondary structure in the B polysaccharide.

CONCLUSION

On the basis of the correlation of serological and NMR spectroscopic data, a working hypothesis has been developed to explain the unique function of sialic acid in the control of the formation of types Ia, II and III Group B streptococcal polysaccharide determinants. The formation of these determinants is achieved by the ability of sialic acid to interact with other remote glycosyl units in the polysaccharides. However, the evidence submitted is insufficient to ignore the possibility of other important factors, not considered in our arguments, involved in what could be a varied and highly complex mechanism. Certainly in addition to hydrogen bonding, the contribution of other means of bonding must be considered in these through-space interactions. Also, because of the limitations of coventional inhibition techniques, the serological evidence supporting the conformational determinant is based on the non-immunogenicity of sialic acid as a whole, and does not consider the possibility that a part of sialic acid could be included in a determinant. This might be important for example, should the carboxylate group of sialic acid function as a small but highly critical part of a determinant. Studies using related oligosaccharides are now in progress, to determine with greater precision the nature of these sialic acid-controlled determinants.

Acknowledgements - We wish to thank Dr. J.B. Robbins for the horse antiserum against the group B meningococcal polysaccharide and Dr. F.E. Ashton for the rabbit antiserum against the group C meningococcal polysaccharide.

REFERENCES

1. H.J. Jennings, Adv. Carbohydr. Chem. Biochem. 41, 155-206 (1983).
2. P. Densen and G.L. Mandell, Rev. Infect. Dis. 2, 817-838 (1980).
3. J.B. Robbins, Immunochemistry, 15, 839-854 (1978).
4. D.L. Kasper and H.J. Jennings, Medical Microbiology, eds. C.S.F. Easmon and J. Jeljaszewicz, Academic Press, New York, 1982, vol. 1, pp. 183-216.
5. M.S. Edwards, D.L. Kasper, H.J. Jennings, C.J. Baker and A. Nicholson-Weller, J. Immunol. 128, 1278-1283 (1982).
6. A.P. Corfield and R. Schauer, Sialic Acids, Chemistry, Metabolism and Function, ed. R. Schauer, Springer-Verlag, New York, Wien, 1982, vol. 10, pp. 5-50.
7. H.J. Jennings, C. Lugowski and D.L. Kasper, Biochemistry 20, 4511-4518 (1981).
8. H.J. Jennings, C. Lugowski, K.-G. Rosell and D.L. Kasper, A.C.S. Symp. Ser., 150, ed. D.A. Brant, American Chemical Society, Washington, D.C., 1980, pp. 161-172.
9. E. Berman, Carbohydr. Res. 118, 9-20 (1983).
10. H.J. Jennings, K.-G. Rosell and D.L. Kasper, Can. J. Biochem. 58, 112-120 (1980).
11. H.J. Jennings, E. Katzenellenbogen, C. Lugowski and D.L. Kasper, Biochemistry 22, 1258-1264 (1983).
12. H.J. Jennings, K.-G. Rosell, E. Katzenellenbogen and D.L. Kasper, J. Biol. Chem. 258, 1793-1798 (1983).
13. W. Reutter, E. Köttgen, C. Bauer and W. Gerok, Sialic Acids, Chemistry, Metabolism and Function, ed. R. Schauer, Springer-Verlag, New York and Wien, 1982, vol. 10, pp. 263-305.
14. L.O. Sillerud, R.K. Yu and D.E. Schafer, Biochemistry 21, 1260-1271 (1982).
15. L.O. Sillerud and R.K. Yu, Carbohydr. Res. 113, 173-188 (1983).
16. R. Kannagi, D. Roelcke, K.A. Peterson, Y. Okada, S.B. Levery and S. Hakomori, Carbohydr. Res. 120, 143-157 (1983).
17. A.K. Bhattacharjee, H.J. Jennings, C.P. Kenny, A. Martin and I.C.P. Smith, J. Biol. Chem. 250, 1926-1932 (1975).
18. H.J. Jennings, A.K. Bhattacharjee, D.R. Bundle, C.P. Kenny, A. Martin and I.C.P. Smith, J. Infect. Dis., Suppl. 136, S78-S83 (1977).
19. R. Spiro and V.D. Bhoyroo, J. Biol. Chem. 249, 5704-5717 (1974).
20. J.E. Sadler, J.C. Paulson and R.L. Hill, J. Biol. Chem. 254, 2112-2119 (1979).
21. G. Spik, B. Bayard, B. Fournet, G. Streker, S. Bougelet and J. Montreuil, F.E.B.S. Lett. 50, 269-299 (1975).

22. P. Fredman, J.E. Mansson, L. Svennerholm and B.E. Samuelsson, F.E.B.S. Lett. 110, 80-84 (1980).
23. S. Ando and R.K. Yu, J. Biol. Chem. 254, 12224-12229 (1979).
24. J. Finne, U. Finne, H. Deagostini-Bazin and C. Goridis, Biochem. Biophys. Res. Commun. 112, 482-487 (1983).
25. J. Finne, M. Leinoren and P.H. Mäkelä, Lancet 355-357 (1983).
26. E.A. Kabat and M.M. Mayer, Experimental Immunochemistry Charles C. Thomas, Springfield, IL., 1961, pp. 241-267.
27. P. Colson, H.J. Jennings and I.C.P. Smith, J. Am. Chem. Soc. 96, 8081-8087 (1974).
28. H.J. Jennings and I.C.P. Smith, Methods Enzymol. 50, 39-50 (1978).
29. H.J. Jennings, E. Katzenellenbogen, R. Roy and D.L. Kasper, manuscript in preparation.
30. D.L. Kasper and H.J. Jennings, to be published.
31. J.Y. Tai, E.C. Gotschlich and R.C. Lancefield, J. Exp. Med. 149, 58-66 (1979).
32. D.L. Kasper, C.J. Baker, B. Galdes, E. Katzenellenbogen and H.J. Jennings, J. Clin. Invest. 72, 260-269 (1983).
33. R.C. Lancefield and E.H. Friemer, J. Hyg. (Cambridge) 64, 191-203 (1966).
34. E.H. Friemer, J. Exp. Med. 125, 381-392 (1967).
35. H.J. Jennings, R. Roy and D.L. Kasper, manuscript in preparation.
36. W.E. Van Heyningen, Nature (London) 249, 415-417 (1974).
37. F.A. Wyle, M.S. Artenstein, D.L. Brandt, D.L. Tramont, D.L. Kasper, P. Altieri, S.L. Berman and J.P. Lowenthal, J. Infect. Dis. 126, 514-522 (1972).
38. H.J. Jennings and C. Lugowski, J. Immunol. 127, 1011-1018 (1981).
39. W. Egan, T.-Y. Lui, D. Dorow, J.S. Cohen, J.D. Robbins, E.C. Gotschlich and J.B. Robbins, Biochemistry 16, 3687-3692 (1977).
40. R.E. Mandrell and W.D. Zollinger, J. Immunol. 129, 2172-2178 (1982).
41. M.R. Lifely, A.S. Gilbert and C. Moreno, Carbohydr. Res. 94, 193-203 (1981).
42. H.J. Jennings, R. Roy and F. Michon, to be published.
43. J.B. Robbins, personal communication.