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Conformational Epitope of the Type III Group B Streptococcus Capsular Polysaccharide

Wei Zou, Roger Mackenzie, Lina Thérien, Tomoko Hirama, Qingling Yang, Margaret A. Gidney, and Harold J. Jennings 2

The protective epitope of the type III group B streptococcal polysaccharide (GBSPIII) is length dependent and conformational. To obtain a more accurate characterization of the conformational epitope, ELISA inhibition and surface plasmon resonance studies were conducted on two GBSPIII-specific mAbs using a large panel of oligosaccharide probes. The results of the studies confirmed that 2 repeating units (RU) is the minimum binding unit and that, while increases in chain length from 2 RU to 7 RU caused further optimization of the epitope, it remained monoclonal. A 3-fold increase in affinity was observed between 7 RU and 20 RU, which, by surface plasmon resonance studies on a Fab, was shown to be due to both further optimization of the individual epitope and the occurrence of multivalency of epitope. The data support our hypothesis that the conformational epitope is an extended helical segment of the GBSPIII. GBSPIII exists mainly in the random coil form, which structurally mimics short oligosaccharide self Ags, but it can infrequently and spontaneously form extended helices. Although not prevalent in GBSPIII, the immune system preferentially selects these helical epitopes because they are unique to the polysaccharide. Contrary to a previously proposed model of GBSPIII binding in which the binding of the first Ab propagates a continuum of helical epitopes, our binding kinetics are consistent only with the helical epitope’s being discontinuous and infrequent. The Journal of Immunology, 1999, 163: 820–825.

The structure of the type III group B streptococcal polysaccharide (GBSPIII) shares extensive homology with other type-specific GBSP (2, 3), and also with human tissue Ags (4–6). Despite structural similarities, all the polysaccharides are both type specific and immunogenic. This is possible because the immune system selects unique polysaccharide epitopes with which to induce high affinity Abs to avoid the problem of mimicry with self Ags (4–7). This hypothesis is supported by the following evidence. Because of failure to define the GBSPIII epitope by classical inhibition techniques using overlapping structures as inhibitors, it was proposed that the epitope was length dependent and conformational in nature (4, 5). This study also established the important role of sialic acid, particularly its carboxylate group, in maintaining the conformational epitope. More recent binding studies (6), using integral GBSPIII RU, confirmed the length dependency of the epitope and established that even 2 RU were required for suboptimal binding. In addition, it was established that the affinity of binding increased with increasing numbers of RU.

We have hypothesized that the immune system selects epitopes situated on extended helical domains of GBSPIII rather than those on the predominant random coil form (7). Because of a lack of symmetry, the random coil form is more structurally related to the short oligosaccharides associated with self Ags. Using nuclear magnetic resonance (NMR) and molecular dynamics studies on GBSPIII and its fragments, we confirmed that GBSPIII was capable of forming extended helices and that sialic acid, although not immunodominant itself, is important in the formation of these helices (7). Here we define the conformational epitope with greater precision by using a much larger repertoire of defined GBSPIII fragments to bind to GBSPIII mAbs, and by employing a number of complementary techniques to study this binding.

Materials and Methods

Oligosaccharides and reagents

Pn14PS was purchased from American Type Culture Collection (ATCC, Manassas, VA). GBSPIII and tetanus toxoid were gifts from Dr. F. Michon of North American Vaccine (Beltsville, MD). HSA and BSA were products of Sigma (St. Louis, MO). All chemicals were purchased from Aldrich Chemical (Milwaukee, WI) and used without further purification.

Preparation of anti-GBSPIII mAbs

Female BALB/c mice, 6–8 wk of age, were immunized intraperitoneally with GBSPIII-TT conjugate (8). Each mouse received 1 μg of polysaccharide in 0.2 ml Ribi complete adjuvant (Ribi ImmunoChem Research, Hamilton, MT) per injection. The mice were boosted on day 21 with an equivalent amount of conjugate vaccine and were trial bled on day 31. The two mice showing the highest Ab titer to the homologous Ag were given two final injections on day 41, an i.p. injection as given previously, and an i.v. injection with the conjugate vaccine in saline (0.1 ml). The fusion was performed 3 days following the last injections. Stimulated spleen cells from the two immunized mice were fused with SP2/0-Ag14 myeloma cells in a ratio of 10:1 in 33% (w/w) PEG 1500. Putative hybrids resulting from hypoxanthine/aminopterin/thymidine (HAT) selection were screened by ELISA against GBSPIII-HSA and Pn14PS-HSA. Those producing Ab of interest were cloned twice by limiting dilution to ensure stability and clonality. Ig subclass was determined on spent supernatant using an ELA mouse mAb isotyping kit (Amersham Canada, Oakville, ON). Clones were expanded as ascites by i.p. injection of 106 hybridoma cells in BALB/c.

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mice 10–14 days following i.p. treatment with 0.5 ml 2,6,10,14-tetramethyl-pentadecane (pristane). Ascitic fluid was tapped 7–14 days postinjection.

**Preparation of Fab fragment**

mAb IB1 was purified from ascites fluid using a protein A column (Pharmacia, Uppsala, Sweden). The purified mAb in PBS at pH 7.4 was concentrated by Centriicon-10 (Amicon, Beverly, MA), and then lyophilized. Fab was prepared from the mAb using an “ImmunoPure Fab Preparation Kit” (Pierce, Rockford, III). Fab was digested into Fab by incubation overnight (15 h) in a buffer containing immunobilized papain in a water bath shaker at high speed. The resultant soluble Fab, Fc fragments, and undigested IgG were separated from the immunobilized papain gel by a separator tube. Then the Fab was separated from the Fc and undigested IgG by using a protein A column. The eluate of the column was collected, dialyzed against PBS at pH 7.4 overnight, and concentrated by Centricon-10.

**ELISA**

Culture supernatant and ascitic fluid were assayed against the HSA conjugates (0.5–1.0 μg/100 μl PBS/well) in 96-well ELISA plates (Linbro, Aurora, OH). Wells were coated at 37°C for 1 h and then washed with PBS-T (0.05% Tween 20) and blocked with 200 ml 1% BSA-PBS for 1 h at room temperature. Samples of culture supernatants or ascites, serially diluted in 1% BSA-PBS, were added and incubated for 1–3 h at room temperature. Following washing with PBS-T, alkaline phosphatase-labeled goat antimouse IgG (Caltag Laboratories, San Francisco, CA) diluted 1:3000 in 1% BSA-PBS was added for 1 h at room temperature. The plates were then washed and developed with p-NPP Phosphate Substrate System (Kirkgaard & Perry Laboratories, Gaithersburg, MD). After 20 min at room temperature, the plates were scanned at 410 nm in a Dynatech (Chantilly, VA) MR 5000 microplate reader.

**Preparation of oligosaccharides and polysaccharides with different chain length**

Oligosaccharides (1 RU to 20 RU) were derived by controlled partial N-deacetylation, enzymatic sialylation, and nitrous acid deamination from Phn14PS (Fig. 1) as previously reported (9). Phn14PS was partially N-deacetylated by base treatment, and the resultant product was enzymatically sialylated at the 3-O position of the terminal galactose residues. The glucosamine residues in the partially N-deacetylated GBSPIII, on treatment with nitrous acid, were converted to 2,5-anhydromannose derivatives. This procedure simultaneously fragmented the polysaccharide and introduced terminal aldehyde group in each of the fragments. Thus, all the fragments made using this degradative procedure contained complete RU of GBSPIII, together with an appendage consisting of a terminal transformed RU (Fig. 1). The fragments were separated on a Superdex 30 column eluted with PBS to afford oligomers from 1 RU to 7 RU. Oligosaccharides representing 1 RU to 4 RU were shown to be pure by spectroscopic analysis. After obtaining the 7 RU fraction, the eluate of the column containing the higher m.w. fractions was also collected. It had an average size of 20 RU, as estimated by comparison with standard dextran samples by HPLC (Hewlett Parkard Series 1100) using a Superose 12 10/30 column (Pharmacia).

For the preparation of fragments larger than 20 RU, a modified procedure was used in which Phn14PS was N-deacetylated to a lesser degree than previously reported (9, 10), and the product was fragmented with nitrous acid before sialylation. Fragments were fractionated on a Biogel A 0.5 column. The fractions were collected and separated into pools with average molecular sizes 25 RU, 28 RU, 42 RU, and 80 RU, estimated by HPLC using a Suprose 12 10/30 column. An oligosaccharide representing two precise repeating units (RU) of GBSP III was synthesized using chemoenzymatic methods previously described (11), and the structure is shown in Fig. 2.

**Conjugation of oligosaccharides (1 RU to 20 RU) to HSA**

The oligosaccharides were conjugated to HSA by a previously described procedure (12). In brief, a solution of oligosaccharide (3 mg), HSA (3.5 mg), and sodium cyanoborohydride (3 mg) in 0.1 M sodium bicarbonate buffer (0.3 ml, pH 8.1) was kept at 37°C for 3–4 days. The progress of conjugation was monitored by analyzing small aliquots from the reaction mixture by HPLC with PBS as eluant. Conjugation was indicated by small shift of the protein peak to a relatively lower K<sub>m</sub> value. All the conjugates were purified on a Biogel A 0.5 column eluted with PBS. Fractions containing the conjugates were pooled, dialyzed, and lyophilized and were analyzed for carbohydrate content by the phenol-sulfuric acid method (13) using GBSPIII as a standard (Table I).

**Conjugation of longer chain GBSPIII fragments to HSA**

These glycoconjugates were prepared by procedures previously described (8). In brief, GBSPIII fragments of different chain lengths were oxidized at C<sub>5</sub>–C<sub>14</sub> of the sialic acid residues with NaOCl to achieve 10–15% oxidation as determined by glc-ms analysis. Conjugation to HSA and purification of the resultant glycoconjugates was performed under the same conditions as described above.

**Direct binding of mAbs to oligosaccharide-HSA conjugates in ELISA**

Microwell plates were coated with 1 μg per well of HSA conjugates in PBS. The plates were then blocked with 0.5% skim milk or 1% BSA in PBS. Serial dilutions of mAbs were applied, and the plates were incubated and washed twice. The Abs were detected with goat anti-mouse IgG (H+L) conjugated to HRP at 1/200 dilution and tetramethylbenzidine (TMB) substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD). After 20 min at room temperature, the plates were scanned at 450 nm in a Dynatech (Chantilly, VA) MR 5000 microplate reader.

**ELISA inhibition of the binding of mAbs IB1 and IA6 to GBSPIII-HSA by GBSPIII fragments**

Competitive inhibition assays were performed following the ELISA procedure as described above, with the following modifications. After the blocking step, 50 μl of oligosaccharide (concentration 2–3.5 mg/ml) in 0.5% BSA/0.02% Tween/PBS buffer was added to the wells, which were serially diluted 2-fold with the same buffer. Then 50 μl of mAb, which was diluted 100× in the same buffer to give an OD of ~1 in the absence of inhibitors, was added, and the mixture was incubated at room temperature for 3 h. The remainder of the procedure was followed as described above. Inhibitory activity is presented as percentage calculated as [OD (no inhibitor) – OD (with inhibitor)/OD (no inhibitor)] × 100%. Inhibition vs log concentration curves were plotted for each inhibitor (see Fig. 3), and the concentrations required for IC<sub>50</sub> were determined from extrapolated curves (Table II).

**SPR Analysis**

Binding kinetics and affinities were determined by SPR using a BIACORE 1000 biosensor system (Biacore, Piscataway, NJ) (14). Glycoconjugates of

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**Table I. Analytical data of HSA-glycoconjugates**

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>1 RU</th>
<th>2 RU</th>
<th>3 RU</th>
<th>4–5 RU</th>
<th>6–7 RU</th>
<th>20 RU</th>
<th>25 RU</th>
<th>28 RU</th>
<th>42 RU</th>
<th>80 RU</th>
<th>GBSPIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Carbohydrate</td>
<td>9.8</td>
<td>11.2</td>
<td>10.0</td>
<td>10.2</td>
<td>10.4</td>
<td>16.2</td>
<td>39.0</td>
<td>45.4</td>
<td>46.5</td>
<td>43.2</td>
<td>44.6</td>
</tr>
</tbody>
</table>

* Measured by H<sub>2</sub>SO<sub>4</sub>-phenol method using GBSPIII as standard.
GBSPIII and its fragments were immobilized on research grade CM5 sensor chips (Biacore) using the amine coupling kit supplied by the manufacturer (Biacore). Immobilizations were conducted in 10 mM sodium acetate (pH 4.5) at conjugate concentrations of 50 \( \mu \text{g/ml} \). The immobilized surface density was ~200 resonance units in each instance. Measurements were conducted in 10 mM HEPES (pH 7.4), 150 mM NaCl, 3.4 mM EDTA at 25°C, and at a flow rate of 20 \( \mu \text{l/min} \). Following IgG or Fab binding, conjugate surfaces were regenerated with 100 mM HCl and a contact time of 6 s. Sensorgram data were analyzed using BIAevaluation 3 software (Biacore).

To determine the capacity of different length oligosaccharides to bind Fab, 50 \( \mu \text{M} \) 1B1 Fab was injected over 2 RU, 6–7 RU, and 20 RU conjugate surfaces. Surface densities were ~200 resonance units for each conjugate, and all three conjugates had very similar degrees of substitution with oligosaccharide (Table I). This Fab concentration is at least 10\(^3\) the \( K_D \) in each instance and should, therefore, result in almost complete saturation of the surface with Fab at equilibrium. The maximum response was used to compare binding stoichiometries.

**Results**

**Conjugation of GBSPIII fragments to HSA**

Conjugation of fragments 1 RU to 20 RU by reductive amination gave conjugates where each saccharide chain was attached to HSA by a single covalent bond. Because they were randomly activated, conjugation of fragments 25 RU to 80 RU (8) resulted in the occurrence of some cross-linking. The analysis of the conjugates is shown in Table I and indicates that conjugates made with fragments 1 RU to 20 RU all had similar saccharide loadings ranging from 9.8 to 16.2%, whereas those made with larger saccharides had much higher saccharide loadings, of the same order as that obtained with the GBSPIII–HSA conjugate.

**Competitive inhibition of the binding of GBSPIII to mAbs by GBSPIII fragments**

For these ELISA inhibition experiments, both mAbs 1B1 and 1A6 were used, and the GBSPIII-HSA conjugate was used as coating Ag. The inhibition curves are shown in Fig. 3 and indicate that, while 1 RU was a very poor inhibitor, inhibition improved dramatically at 2 RU and improved further at 3 RU. The inhibitory properties of 2 RU, whether obtained by degradative methods (2 RU) or by synthesis (2 RU\(^c\)) (Fig. 2), were the same, thus demonstrating that the residual terminal oligosaccharide appendage of the former did not add to its inhibitory properties. Further increases in saccharide length from 3 RU to 7 RU did not increase the inhibitory properties to any extent. However, substantial increases in inhibition were obtained when larger fragments were employed. The inhibitory power of these larger fragments (25 RU to 80 RU) increased sequentially with increasing length, GBSPIII being the most potent inhibitor.

The binding affinity of GBSPIII fragments to the mAbs was also evaluated by comparing their IC\(_{50}\) data. These results are shown in Table II and again confirm the dramatic leap in 1B1 and 1A6 affinity that accompanies progression from 1 RU to 2 RU. For 1B1, affinity maximizes at 3 RU and remains constant up to 7 RU. In the case of mAb 1A6, inhibition maximized at 4–5 RU and also remained constant up to 7 RU. This is consistent with the binding site of 1A6 being longer than that of 1B1. These results also indicate that the inhibitory activity of GBSPIII is ~200-fold better than that of fragments of 2 RU to 7 RU.

**Table II.** IC\(_{50}\) of oligosaccharides in the binding of GBSIII-PS to mAbs 1B1 and 1A6\(^a\)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>1 RU</th>
<th>2 RU(^b)</th>
<th>3 RU</th>
<th>4–5 RU(^c)</th>
<th>6–7 RU(^c)</th>
<th>25 RU</th>
<th>42 RU</th>
<th>80 RU</th>
<th>GBSPIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B1 (\mu\text{g/ml})</td>
<td>293.0</td>
<td>19.0</td>
<td>25.4</td>
<td>14.3</td>
<td>13.6</td>
<td>15.5</td>
<td>3.5</td>
<td>1.1</td>
<td>0.7</td>
</tr>
<tr>
<td>(\mu\text{M})</td>
<td>152.4</td>
<td>9.5</td>
<td>8.6</td>
<td>3.7</td>
<td>3.0</td>
<td>2.9</td>
<td>(^d)</td>
<td>(^d)</td>
<td>(^d)</td>
</tr>
<tr>
<td>1A6 (\mu\text{g/ml})</td>
<td>1221.7</td>
<td>287.4</td>
<td>248.2</td>
<td>89.6</td>
<td>50.9</td>
<td>69.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>(\mu\text{M})</td>
<td>635.4</td>
<td>144.1</td>
<td>85.4</td>
<td>23.1</td>
<td>11.6</td>
<td>12.9</td>
<td>(^d)</td>
<td>(^d)</td>
<td>(^d)</td>
</tr>
</tbody>
</table>

\(^a\) Data obtained from Fig. 3.

\(^b\) Obtained by synthetic procedure (see Fig. 2).

\(^c\) A mixture of two oligosaccharides in a ratio of approximately 1:1.

\(^d\) Due to the multivalent nature of the GBSPIII, the molar concentrations were not meaningful.
Binding kinetics and affinities of GBSPIII fragments by SPR analysis

The binding of anti-GBSIII mAb 1B1 and its Fab fragment to HSA conjugates of GBSPIII fragments were investigated by SPR. The binding of 1B1 Fab fragments to immobilized oligosaccharide conjugates showed good fitting to a 1:1 interaction model. The simultaneous fitting of the association and dissociation data of individual sensorgrams shown in Fig. 4 for the 20 RU conjugate is representative of that observed with all conjugates. Association and dissociation rate constants for 1B1 Fab binding to the complete glycoconjugate panel were derived by this fitting procedure (Table III). For 1B1, the pattern of affinities exhibited by the saccharide fragments with increasing size is in good agreement with that obtained by other approaches. The binding of 1 RU surfaces was very weak; Fab binding was not detectable and the level of IgG binding was significant only at high surface densities (4000 resonance units). Epitopic stabilization appears to occur at 2 RU with the $K_a$ remaining virtually constant from 2 RU to 7 RU. A gradual 3-fold increase in affinity, attributable to equal contributions from a faster $K_a$ and slower $K_d$, occurs with increasing saccharide size from 6 RU to 20 RU. Further increases in chain length did not lead to much improved binding. Thus, there is dramatic epitopic stabilization at 2 RU with a further significant stabilization between 6 RU and 20 RU.

To obtain information on the length of saccharide required for epitope multivalency to occur, a saturation binding experiment was performed using 1B1 Fab and the same GBSPIII fragment surface densities as those used in the above kinetic experiment. Saturation binding experiments provide values for surface binding capacities in resonance units and allow for the calculation of binding stoichiometries. The three sensorgrams shown in Fig. 5 reveal identical steady state binding levels for 2-RU and 6- to 7-RU surfaces of the same density ($R_{\text{max}} = 148$ resonance units) and that a 20-RU surface of the same density had a 3-fold greater capacity for Fab binding ($R_{\text{max}} = 486$ resonance units). This is consistent with a Fab-to-Ag binding stoichiometry of 1:1 for the 2-RU and 6- to 7-RU surface and 3:1 for the 20-RU surface and indicates that epitope multivalency occurs between 6–7 RU and 20 RU.

Although only qualitative because of varying and unknown ratios of monovalent and bivalent binding, the 1B1 IgG binding data provided a relative measure of binding avidity. An increase in the avidity of binding results in a slower dissociation of the Ag-Ab complex. With all conjugates, slower dissociation rates were observed for IgG binding than for Fab binding (Table III). However, the differences between the dissociation rates for IgG and Fab increased with increasing saccharide length, indicating a higher proportion of bivalent binding at higher Ag multivalency.

### Discussion

Previous studies (6, 8) had identified the length dependency of the GBSPIII epitope and had established that 2 RUs were required for even suboptimal binding. Our inhibition results on mAbs 1B1 and 1A6 using GBSPIII fragments (n RU) were similar (Fig. 3), thus confirming that both mAbs has a specificity similar to that of the previously defined conformational epitope (6, 8). Even though each of the fragments used in our studies contained an additional terminal oligosaccharide appendage, they were designated on the basis of the number of complete RUs that they contained. That this designation had relevance in addition to convenience was established in inhibition experiments, the result of which demonstrated that the appendage made no contribution to the inhibitory properties of the fragments.

Having established 2 RU as the minimum binding structure, we designed additional experiments to study in more detail factors behind the increasing affinity of GBSPIII with increasing length (6, 8). Using mAbs 1B1 and 1A6 and a more extensive series of well-defined chemically derived GBSPIII fragments, we attempted to answer two questions that were not addressed in previous studies (6, 8): 1) at what fragment length does the conformational epitope

| Table III. Kinetic and affinity constants for 1B1 Fab binding to neoglycoconjugates |
|---------------------------------|------------------|------------------|
| $K_a$ (M$^{-1}$s$^{-1}$) | $K_d$ (s$^{-1}$) | $K_I$ (M) |
| 80 RU | 1.9 x 10$^8$ (±5.8)$^*$ | 1.3 x 10$^{-3}$ (±10.8) | 6.5 x 10$^{-7}$ (±16.9) |
| 42 RU | 2.1 x 10$^8$ (±5.7) | 1.4 x 10$^{-3}$ (±6.1) | 6.7 x 10$^{-7}$ (±7.9) |
| 28 RU | 2.5 x 10$^8$ (±10.8) | 1.5 x 10$^{-3}$ (±6.1) | 6.1 x 10$^{-7}$ (±16.4) |
| 25 RU | 1.4 x 10$^8$ (±11.2) | 1.4 x 10$^{-3}$ (±9.3) | 9.5 x 10$^{-7}$ (±2.7) |
| 20 RU | 1.3 x 10$^8$ (±9.2) | 1.7 x 10$^{-3}$ (±5.0) | 1.3 x 10$^{-6}$ (±15.4) |
| 6–7 RU | 6.4 x 10$^8$ (±5.6) | 2.6 x 10$^{-3}$ (±2.9) | 4.0 x 10$^{-6}$ (±6.8) |
| 3 RU | 8.1 x 10$^8$ (±10.2) | 3.0 x 10$^{-3}$ (±5.7) | 3.7 x 10$^{-6}$ (±16.7) |
| 2 RU | 7.8 x 10$^8$ (±6.2) | 3.6 x 10$^{-3}$ (±3.9) | 4.6 x 10$^{-6}$ (±8.0) |

$^*$ Numbers in parentheses are % SE.
optimize, and 2) at what length of saccharide does epitope multivalency occur? ELISA inhibition of the binding of GBSPIII to mAbs 1B1 and 1A6 using GBSPIII fragments demonstrated that the initial formation of the conformational epitope occurs at 2 RU. A small increase in inhibition occurs from 2 RU to 3 RU, with little subsequent increase in inhibition from 3 RU to 6–7 RU. The inhibition results using mAb 1A6 were very similar. These data are consistent with the presence of only one conformational epitope from 2 RU to 7 RU, and with little further optimization of epitope in this region. The above data were confirmed by studies on 1B1 binding to GBSPIII-HSA by SPA. Further increases in inhibition and binding with fragments larger than 7 RU were initially attributed to epitope multivalency, but SPR data obtained using 1B1 Fab clearly demonstrated that optimization of epitope was still a factor even at this length of saccharide.

Because of IgG binding, both the SPR and inhibition studies could not distinguish between the contribution of epitope multivalency and epitope optimization to the increasing affinity with increasing length of GBSPIII. However, valency effects were eliminated by binding 1B1 Fab to GBSPIII-HSA, and the results up to 7 RU thus obtained were again in excellent agreement with the inhibition data. The SPR data showed that oligosaccharides in the 2 RU to 7 RU range were monovalent with respect to epitope presentation and bound to Fab with a \( K_D \) of \( \sim 4 \mu M \). The IC\textsubscript{50} values for these monovalent Ags, which should be equivalent to the \( K_D \) of their interactions with Ab, were 4 \( \mu M \), based on an epitope size of 3 RU (Table III). However, the SPR data also demonstrated that affinity still increased 3-fold from 7 RU to 20 RU, with little increase in affinity with increasing length beyond this point. Thus, optimization of the conformational epitope occurs between 7 RU and 20 RU and is a factor, together with multivalency of epitope, in the increasing affinity of 1B1 IgG with increasing GBSPIII length. Interestingly, saturation-binding SPR experiments using 1B1 Fab indicate that multivalency of epitope also occurs in the same region (7 RU to 20 RU).

The characteristics of the GBSPIII conformational epitope as defined in the above experiments are similar to those associated with the immune response to the capsular polysaccharides of group B Neisseria meningitidis (15) and type 14 Streptococcus pneumoniae (10, 16). Because they all exhibit structural mimicry with self Ags, which usually consist of short oligosaccharides, the immune system selects length dependent epitopes unique to the polysaccharides for generating high affinity Abs. We have hypothesized (7) that these epitopes are located on extended helical domains of the polysaccharides rather than on its random coil form. Because of lack of symmetry, the random coil form is structurally related to short oligosaccharide self Ags. Although we have no direct evidence for the existence of extended helical epitopes on GBSPIII or Pn14PS, nuclear magnetic resonance and molecular dynamics studies indicate that both the above polysaccharides (17) and the group B meningococcal polysaccharide (18) are capable of forming extended helices. Additionally, there is a precedent for this type of epitope because there is strong evidence, based on x-ray crystallographic studies (18), that the extended epitope of the group B meningococcal polysaccharide is helical. Interestingly, both the length dependency of the GBSPIII epitope and its distribution are similar to those found for Pn14PS and the group B meningococcal polysaccharide. Studies on the immune response to Pn14PS conjugates in rabbits are consistent with the length-dependent epitope forming at 4 RU and persisting as a single epitope up to \( \sim 20 \) RU (10). Similarly, the \( \alpha_2,8 \)-polysialic acid helical epitope of group B \( N. meningitidis \) requires \( \sim 10 \) sialic acid residues to form, and the helix is not duplicated until there are \( \sim 40 \) linear sialic acid residues present (18).

Wessels et al. (6) proposed a model of Ab binding to GBSPIII in which the binding of the first Ab induces a second epitope in the same chain, which by means of a process of epitope propagation results in the accumulation of Abs on previously bound chains rather than on naive chains. The SPR data reported here do not support this model of Ab binding to GBSPIII. Such a model would not give good fitting of Fab association data to a 1:1 interaction since the epitope density would remain constant throughout the association at nonsaturating concentrations of Fab. Our model of binding requires that helical epitopes are intermittently located between random coil segments of GBSPIII. This pattern of epitope distribution is not conducive to an induction process that would require the formation of contiguous helices. In our model, epitopes form spontaneously, and the number of epitopes per polysaccharide chain would be more restricted. This unusual Ag characteristic may be reflected in the association rate constants for 1B1 Fab binding to Ag, which are, to our knowledge, the lowest reported for Ag-Ab interactions. Values in the range of \( 10^4 \)–\( 10^5 \) M\(^{-1}\) s\(^{-1}\) are typical for these interactions whereas those reported here for 1B1 Fab are \( 2 \times 10^5 \) M\(^{-1}\) s\(^{-1}\) for the longer oligosaccharides and slightly lower for the shorter chain Ags. The reason for these unusually slow rates may lie in the dynamic nature of the Ag. The extended helical conformation represents a minor proportion of the total polysaccharide, with helices forming intermittently and reverting to random coil. Despite the slow association rate constant for the interaction, a slow dissociation constant contributes to an overall affinity that is unusually high for an anti-carbohydrate Ab. With a \( K_D \) of \( \sim 0.65 \mu M \), 1B1 Fab is at the upper end of the affinity range for protein-carbohydrate interactions. For example, the affinities of Abs that are specific for the Salmonella serogroup B O-polysaccharide and the human blood group A determinant have been determined, by SPR, to be 8 \( \mu M \) (19) and 290 \( \mu M \) (20), respectively.

In our model of binding, we would predict one helical epitope per Fab bound, which would be consistent with the SPR data and the x-ray crystallographic data obtained on the Fab binding of the conformationally related group B meningococcal polysaccharide (18). In these circumstances, bivalent Ab binding to one saccharide chain could not readily occur, especially in the shorter chains (3 RU to 6–7 RU) where only one binding site resides. Any contribution from steric hindrance in addition to the conformational length requirement in restricting binding to a single Fab at lengths as long as 7 RU (\( \sim 200 \AA \)) is unlikely, but cannot be ruled out on our evidence. The SPR data for 1B1 IgG binding to the panel of glycoconjugates showed that bivalent binding is both intersaccharide and intrasaccharide in nature. Avidity effects were observed even with conjugates presenting short chain oligosaccharides with single epitopes. This avidity could arise only through intersaccharide binding. With conjugates presenting longer chain oligosaccharides, the avidity effects were much more pronounced, indicating an additional contribution from intrasaccharide bivalent binding.

References


