Preliminary Comparison of the Epitope Maps of Ligands Directed Towards Monoclonal Antibody SYA/J6 using STD-NMR.

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Introduction

High resolution details of carbohydrate–protein interactions have been used to design higher affinity univalent carbohydrate ligands in a model system. A monoclonal antibody, SYA/J6, that was produced against the O-polysaccharide (LPS) of the Shigella flexneri variant Y lipopolysaccharide. The biological repeating structure is shown below and the residues are identified as A-B-C-D.

Crystal structures 1 of SYA/J6 with bound ligands (an ABCDA' pentasaccharide and trisaccharide 2) detailed the position of the optimal trisaccharide epitope 1, residues BCD of the LPS. Analysis of binding site contacts made to 1 and the higher affinity deoxygenated trisaccharide 2 indicated that they bound to the antibody in different modes. Although no co-crystal structure data for SYA/J6 and 3 is available, it has been postulated that this higher-affinity ligand would bind in the same mode as 1 since the exchange of a hydroxyl for a chlorine atom is isosteric and of comparable electronegativity (Figure 1).4

The crystal structure of the complex of SYA/J6 with 1 showed the methyl groups of the 2-acetamido of the D-GlcNAc D-residue and C-6" of the L-Rha-GlcNac C-residue (figure 2).5 This ligand also displayed a higher affinity for the receptor than the native 1. To illustrate that the pre-organization was responsible for the observed affinity increases, acyclic ‘control’ derivatives of 4 were synthesized (5 and 6, Figure 2). We also hypothesized that combining the functional group modifications of 2 and 3 with the tethering methodology of 4, could produce high affinity ligands for SYA/J6 (compounds 7 and 8, Figure 2).6

Table 1. Titration microcalorimetry of 1 to 3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>K_a (mM)</th>
<th>ΔT_H</th>
<th>ΔT_S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1 x 10^6</td>
<td>-6.8 ± 0.2</td>
<td>-3.9 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>2.5 x 10^5</td>
<td>-8.5</td>
<td>-8.1</td>
</tr>
<tr>
<td>3</td>
<td>1.1 x 10^6</td>
<td>-8.1</td>
<td>-6.3</td>
</tr>
</tbody>
</table>

Table 2. Titration Microcalorimetry of compounds 4 through 8.

<table>
<thead>
<tr>
<th>Compound</th>
<th>K_a (mM)</th>
<th>ΔT_H</th>
<th>ΔT_S</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.5 ± 0.05 x 10^6</td>
<td>-8.3 ± 0.1</td>
<td>-4.2 ± 0.05</td>
</tr>
<tr>
<td>5</td>
<td>2.0 x 10^6</td>
<td>-5.8</td>
<td>-3.5</td>
</tr>
<tr>
<td>6</td>
<td>2.9 x 10^6</td>
<td>-6.0</td>
<td>-3.1</td>
</tr>
<tr>
<td>7</td>
<td>2.6 x 10^6</td>
<td>-8.6 ± 0.15</td>
<td>-4.4 ± 0.1</td>
</tr>
<tr>
<td>8</td>
<td>4.3 x 10^5</td>
<td>-7.4</td>
<td>-5.8</td>
</tr>
</tbody>
</table>

We now report preliminary data acquired using saturation transfer difference NMR (STD-NMR) methods to resolve whether or not these compounds, or subgroups of them, bind in similar modes. Since the substitution of a hydroxyl by a chlorine atom is a conservative modification we expect compounds 1 and 2 to bind in a similar manner to the protein. By extension, cyclic 4 and cyclic-chloro-7 should also bind in the same mode. We hope to use this technique to understand why compound 8 bound so poorly, and comment on the non-additivity of pairing functional group modifications with intramolecular tethering.

STD-NMR Spectra

(A) The 1D NMR spectrum of SYA/J6 with a standard pre-saturation sequence for HOD suppression. Despite the 13 mM IgG concentration one can still observe the broad resonances of the protein. (B) The reference spectrum of 1 (100-fold excess) and SYA/J6. The on and off resonance pulses are now 30 ppm. The T_r filter was used to remove the protein signals. (C) The STD spectrum of 1 and the antibody SYA/J6. Notice that small molecule impurities seen in spectrum (A) have been completely subtracted.

Results and Conclusions

Compounds 1 and 3 clearly show different intensities in their STD spectra for comparable protons. This disproves our hypothesis that these two compounds bind in a similar manner. It has been postulated that there is a stabilizing interaction between the chlorine and Tyr L32,1 and it could be this interaction that causes 3 to bind in a different mode.

Acknowledgements

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References