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

Robert S. McGavin, Thomas Peters, and David R. Bundle*

Alberta Ingenuity Centre for Carbohydrate Science, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada,T6G 2G2

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 ABCD

-[-2)-α-L-Rha*p*(1-2)-α-L-Rha*p*(1-3)-α-L-Rha*p*(1-3)-β-D-GlcNAc*p*(1-]$_n$-

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**[2] indicated that they bound to the antibody in different modes. Although no co-crystal structure data for SYA/J6 and **3**[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]

Figure 1

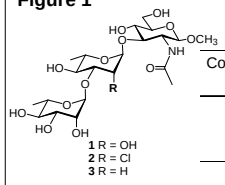


Table 1. Titration microcalorimetry of **1** to **3.**

Compound	K_A (mol^{-1})	ΔG ($Kcal/mol$)	ΔH ($Kcal/mol$)	$-T\Delta S$ ($Kcal/mol$)
1	1.1 x 10^5	-6.8 ± 0.2	-3.9 ± 0.1	-2.9 ± 0.1
2	2.5 x 10^6	-8.5	-8.1	-0.5
3	1.1 x 10^6	-8.1	-6.3	-1.8

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-rhamnosyl B-residue pointing towards bulk solvent upon binding. Thus, a pre-organized ligand was developed using a β-alanine intramolecular tether that spanned the B and D residues as seen in **4** (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]

Figure 2

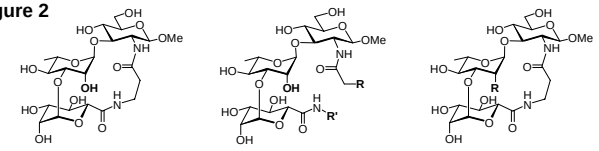


Table 2. Titration Microcalorimetry of compounds **4** through **8**

Compound	K_A (mol^{-1})	ΔG ($kcal/mol$)	ΔH ($kcal/mol$)	$-T\Delta S$ ($kcal/mol$)
4	1.5 ± 0.05 x 10^6	-8.3 ± 0.1	-4.2 ± 0.05	-4.1 ±0.05
5	2.0 x 10^4	-5.8	-3.5	-2.3
6	2.9 x 10^4	-6.0	-3.1	-2.9
7	2.6 x 10^6	-8.6 ± 0.15	-4.4 ± 0.1	-4.2 ± 0.05
8	4.3 x 10^5	-7.4	-5.6	-1.8

Isothermal Titration Microcalorimetry

• Acyclic derivatives **5** and **6** show that pre-organization is responsible for the increased activity of cyclic **4**.

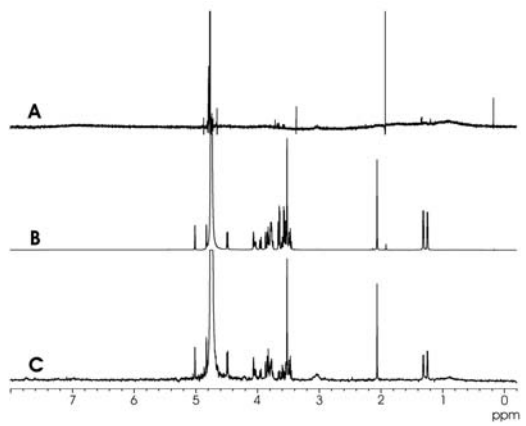
• The affinity of cyclic 2"-deoxy-chloro trisaccharide **7** was the highest of all derivatives, though no additivity was observed for the pairing of tethering with functional group replacement.

• The 2'-deoxy cyclic trisaccharide **8** bound with much reduced affinity, even less than its parent trisaccharides, namely cyclic **4** and deoxy **2**.

We now report preliminary data acquired using saturation transfer difference NMR (STD-NMR)[7] methods to resolve whether or not these compounds, or subgroups of them, bind in similar modes. Since the substitution for 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.

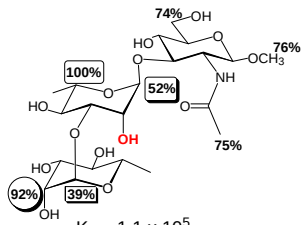
STD-NMR Spectra

Figure 3



(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_{1r} 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.

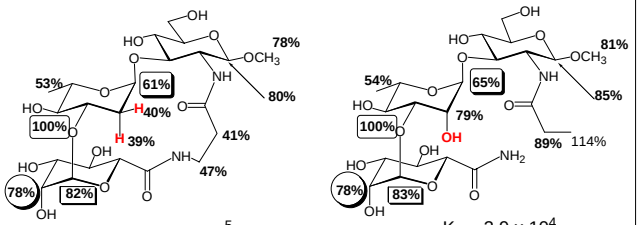
Results and Conclusions



K_A = 1.1 x 10^5 **1**

K_A = 1.1 x 10^6 **3**

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.



K_A = 4.3 x 10^5 **5**

K_A = 2.9 x 10^4 **8**

Compounds **6** and **7** have matching epitope maps (± 5% STD intensity) that suggests the tether is incompatible with the preferred mode for binding of deoxygenated **7.** Acyclic derivative **7** with its hydroxyl at the C-2' position proves that the tether, or components of it, must make a physical or electronic contact with the protein thus preventing **6** penetrating as far into the binding site as it's acyclic parent **2**.

When the STD spectra are acquired for all compounds, we should be able to comment on the implications of functional group modification, intramolecular pre-organization, and the combination of the two.

Acknowledgements

Financial support for this work was provided by research grants from the Natural Sciences and Engineering Research Council of Canada (NSERC), the University of Alberta, and NSERC, AHFMR, and ARC postgraduate scholarships to R.S. McGavin. Special thanks go to Ms. J. Sadowska for antibody preparation and Ms. M. Lockyer for preparation of SYA/J6 for NMR

References

1) N.K. Vyas, M.N. Vyas, M.C. Chervenak, M.A. Johnson, B.M. Pinto, D.R. Bundle, F.A.Quiocho, *Biochemistry*, 2002, **41**, 13575.

2) H.R. Hanna, D.R. Bundle, *Can. J. Chem.*, 1993, **71**, 125.

3) F-I. Auzanneau, D.R. Bundle, *Carbohydr. Res.*, 1993, **247**, 195.

4) A. Maradufu, A. S. Perlin, *Carbohydr. Res.*, 1974, **32**, 93.

5) R.S. McGavin, R.A.Gagne, Chervenak, M.C., D.R. Bundle, *Org. Biomol.* Chem, in press.

6) R.S. McGavin, D.R. Bundle, *Org. Biomol. Chem.*, in press.

7) (a) M. Mayer, B. Meyer, *J. Am. Chem. Soc.*, 2001, 123, 6108. (b) B. Meyer, T. Peters, *Angew. Chem. Int. Ed.*, 2003, 42, 864.