Thermodynamic and Conformational Implications of Glycosidic Rotamers Preorganized for Binding

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Branched trisaccharides have been synthesized with six different intramolecular tethers (Alibés and Zhang, manuscripts in preparation) and in four cases the resulting macrocycle constrains the conformation of the oligosaccharide so that it resembles the bound state of the complex with antibody as determined by crystallography.[1,2] Measurement of the binding constants of the constrained trisaccharides **4**−**7** indicates free energy changes ($\Delta\Delta G°$) relative to the trisaccharide **1** no larger than ±0.5 kcal mol^{-1}, despite preorganization by restriction of flexibility about one glycosidic linkage. These data suggest that interresidue flexibility is not a major contributor to the weak association that characterizes oligosaccharide−protein interactions,[3] and imply that other factors such as solvent reorganization[4,5] modulate $\Delta G°$. Stereoelectronic effects at the glycosidic linkage[6] may also result in an inherent level of conformational constraint (preorganization).

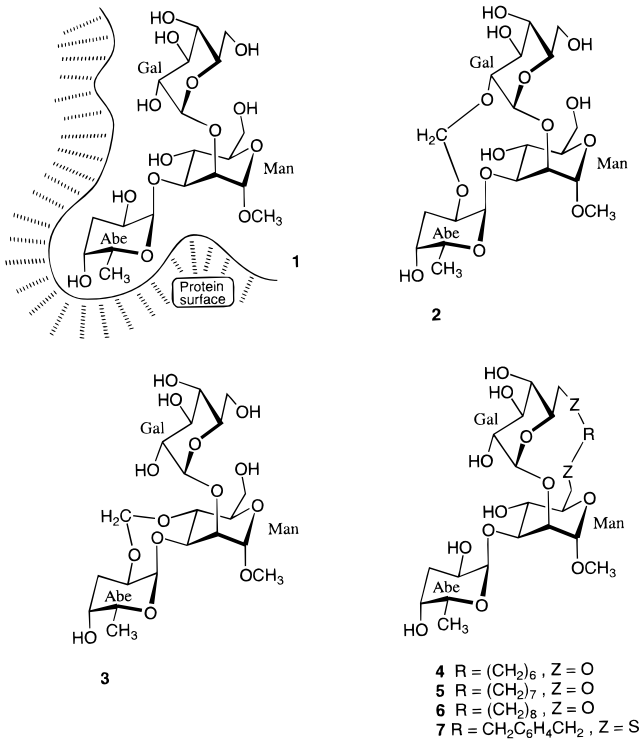

Figure 1. Schematic of the binding surface with trisaccharide 1, and structures of the tethered ligands 2−7.

Carbohydrate antigens exhibit disassociation constants (K_D) for lectin and antibody binding sites that typically fall in the milli- to micromolar range, and almost without exception oligosaccharides fail to reach nanomolar K_D.[3,7] Exceptions are found in oligovalent ligands[8] such as the recently reported divalent inhibitor of *Streptococcus suis* adhesion,[9] where avidity effects are exploited. Other low molecular weight ligands, such as peptides or steroids, show significantly higher binding affinities for proteins as exhibited by nanomolar K_D with antibodies.[10,11] The selection of a single bound conformation with its consequent freezing or restriction of inter-saccharide bond rotamers has been estimated to carry a conformational entropy penalty as large as 1−2 kcal mol^{-1} per rotor.[3] Other estimates for freezing single bond rotamers are more conservative at ∼0.6 kcal mol^{-1} per torsion.[12,13]

Table 1. Thermodynamics[a] of Antibody−Oligosaccharide Interaction (kcal mol^{-1}) for Native and Tethered Trisaccharides at 25.2 °C

ligand	K_A (M^{-1})	$\Delta G°$	$\Delta H°$	$T\Delta S°$
1	$(1.60 \pm 0.28) \times 10^5$	-7.10 ± 0.10	-6.75 ± 0.38	0.34 ± 0.48
4	$(1.30 \pm 0.08) \times 10^5$	-6.97 ± 0.03	-8.76 ± 0.18	-1.79 ± 0.21
5	$(1.30 \pm 0.09) \times 10^5$	-6.98 ± 0.04	-8.16 ± 0.15	-1.18 ± 0.19
6	$(1.47 \pm 0.01) \times 10^5$	-7.04 ± 0.01	-8.58 ± 0.04	-1.54 ± 0.05
7	$(3.85 \pm 0.05) \times 10^5$	-7.60 ± 0.01	-6.95 ± 0.05	0.65 ± 0.06

[a] Experimental conditions given in Supporting Information.

Several attempts have been made to exploit conformational entropy by either locking in or biasing conformations toward the bioactive conformation.[14−17] Related to this study, a tethered trisaccharide has been designed and synthesized for studies of lectin binding.[18] In this report we describe tethering studies conducted with a branched trisaccharide epitope that binds to its monoclonal antibody with an affinity (K_A) of 1.6×10^5 M^{-1} and for which a significant number of high-resolution crystal structures[1,2,19] and thermodynamic data are available.[20]

The binding site of the monoclonal antibody Se 155.4 is dominated by aromatic amino acid residues and buries the 3,6-dideoxy-D-*xylo*hexose (Abequose, Abe) of the trisaccharide epitope **1** (Figure 1, Supporting Information). The mannose and galactose residue are partially solvent exposed, although both are postulated to make hydrogen bonds to the antibody.[1,2] To date,

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Table 2. Experimental NOE-Based and MD-Derived Distances in Å[a]

	tether type and length	Gal-H1: Man-H2	Abe-H1: Man-H3	Gal-H1: Man-H1	Man-H1: Gal-H5	Gal-H1: Abe-H5	Gal-H1: Abe-H3$_{ax}$
1	none[b]	2.2 (2.3)	overlap	3.5[c] (3.2)	2.5 (2.4)	2.7 (2.5)	4.0 (3.9)
2	AbeO2-GalO2: (CH_2)	2.1	2.7	*d*	2.3	*e*	*e*
3	AbeO2-ManO4: (CH_2)	2.3	overlap	3.9	2.6	3.1	4.3
4	GalO6-ManO6: $(CH_2)_6$	2.2 (2.2)	2.2 (2.3)	3.2 (3.4)	2.6 (2.3)	2.8 (2.3)	*e*
5	GalO6-ManO6: $(CH_2)_7$	2.2	2.3	overlap	2.6	2.8	*e*
6	GalO6-ManO6: $(CH_2)_8$	2.2	2.4	3.6	2.6	2.8	*e*
7	GalS6-ManS6: $CH_2(C_6H_4)CH_2$	2.2 (2.2)	2.3 (2.3)	3.5 (3.6)	2.5 (2.5)	2.6 (2.5)	4.2 (4.0)
	bound conformer II[2]	2.1	2.4	3.3	2.7	3.0	4.0

[a] Values in parentheses are from restrained MD calculations, except for **1** which was subjected to unrestrained MD. [b] A Man-1:H2 distance of 2.44 Å was used for distance calculations except when this correlation is obscured by spectral overlap. Then a Gal-H1:H2 of 2.55 Å was used as a reference in **1**. [c] Not available in **1** (too close to diagonal); the value is from an analogue pentasaccharide.[30] [d] The cross-peak is very weak and too close to the diagonal for reliable quantification. [e] NOE not observed. [See Supporting Information for details of MD calculations.]

in all crystal structures the Abe-Man glycosidic linkage adopts a conformation (72°/104°)[21] close to that predicted by potential energy calculations. Depending on the complex, the Gal-Man glycosidic linkage exists in one of two conformations (I, 104°/89° and II, 77°/144°), which are related by a shift in the φ/ψ torsional angles.[2] In the complex a hydrogen bond between Abe O-2 and Gal O-2 is either direct (conformation I)[1] or mediated by a water molecule (conformation II),[2] while the hydrogen bonding pattern to the galactose residue is also modified.[2,19] If the trisaccharide is tethered by acetals (**2** or **3**), the conformation of the trisaccharide is significantly distorted, mostly about the Abe-Man linkage, and bioactivity is destroyed, since neither bound conformation can be adopted. However, since both primary hydroxyl groups of trisaccharide **1** are solvent exposed and positioned at least 4−6 Å from the protein (Figure 1, Supporting Information), the introduction of tethers between the Man C-6 and Gal C-6 atoms yields macrocyclic structures **4**−**7** that are constrained to a series of conformations similar to bound conformation II. For example, trisaccharide **4** samples a range of torsional angles (ϕ 50−100°; ψ 130−160°) compared to those populated by the untethered trisaccharide **1** (ϕ 30−110°; ψ 50−200°) [Figure 2, Supporting Information]. Conformation I cannot be populated and the conformation of the Abe-Man linkage is unaffected. The resultant affinity is either slightly less (**4**−**6**) or greater (**7**) than that seen for **1** (Table 1).

The conformations of the trisaccharides **1**−**7** were investigated by NMR methods that included ^{1}H and ^{13}C chemical shift assignments, dipolar couplings[22] (quantitative NOEs), and scalar coupling constants [for experimental details see Supporting Information]. Homonuclear NOEs were divided into three classes (strong, medium, and weak) and the corresponding distance constraints were employed for restrained molecular dynamics simulation.[23,24] Agreement between observed and simulated conformations for trisaccharides **4**−**7**, i.e. interproton distances (Table 2) and torsional angles (Figure 2, Supporting Information) is consistent with the population of a narrow set of conformers that also coincide with bound conformation II, a conclusion supported by the thermodynamic data (Table 1). Conformers trapped in the wrong configuration would be expected to show decreases in enthalpies and entropies of binding, whereas $\Delta\Delta H°$ exhibits small exothermic changes.

Titration thermodynamics for the native trisaccharide **1** and macrocyclic analogues **4**−**7** reveals small changes in $\Delta G°$ with compensatory changes in enthalpy and entropy (Table 1). While the trisaccharides with an oligomethylene ether tether (**4**−**6**) show weaker binding, an entropic penalty is almost offset by enthalpy. The entropy/enthalpy compensation is virtually reversed for the aryl dithioether **7**. Since the NMR derived interproton distances (Table 2) and restrained MD simulations for all four macrocyclic analogues are similar, the origin of the thermodynamic differences is attributed to the chemical nature of the tethers. Increased water structure about the more hydrophobic oligomethylene tether could be anticipated to contribute to the exothermicity of $\Delta H°$ (hydrophobic effect).[25,26] On the other hand, aryl groups are known to be capable of polar interactions[27] and hydrogen bonding with water.[28]

Even though the origin of these compensatory enthalpy/entropy changes may be the subject of conjecture, it is clear that a significant reduction of torsional flexibility (Figure 2, Supporting Information) has failed to produce entropic gains that result in $\Delta\Delta G°$ approaching the magnitude predicted for freezing two single bond rotamers.[3,13] Based on our results, the preordering of the glycosidic bond conformation is an approach in the search for tight binding sugar-based antagonists, which by itself seems unlikely to facilitate the design of carbohydrate-based therapeutics.[16,29] The absence of large impacts on ΔH and ΔS also implies that oligosaccharides may display a restricted range of conformations, as originally proposed by Lemieux.[6]

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Supporting Information Available: Figure giving the bound conformation I of trisaccharide **1** complexed with monoclonal antibody Se 155.4 and Figure 2 showing the rotamer populations for the Gal-Man linkage in **1** and the tethered trisaccharide **4** as well as experimental details and references (5 pages, print/PDF). See any current masthead for ordering information and Web access instructions.

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