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# Thermodynamics of lectin–sugar interaction: Binding of sugars to winged bean (*Psophocarpus tetragonolobus*) basic agglutinin (WBAI)

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***Abstract*** Combining site of WBAI is extended and encompasses all the residues of blood group A-reactive trisaccharide [GalNAcα3Galβ4Glc]. Though both of the fucose residues of A-pentasaccharide [GalNAcα(Fucα2)3Galβ(Fucα3)4Glc] do not directly interact with the combining site they thermodynamically favour the interaction of GalNAcα3Galβ4Glc part of the molecule by imposing a sterically favourable orientation of the binding epitope viz. GalNAcα3Galβ4Glc of the saccharide. Binding of sugars is driven by enthalpy and is devoid of heat capacity changes. This together with enthalpy-entropy compensation observed for these processes underscore the importance of water reorganization as being one of the principal determinant of protein-sugar interactions.

Recognition is a key event in many biological phenomena. It is also an initiation step in numerous processes, based on cell-cell interactions, such as fertilization, embryogenesis, organ formation, immune and microbial defences, etc. (1). Carbohydrates perched at the surface of the cells provide cells with their individuality and recognition patterns that generally play a crucial role in the day to day life of an ordinary cell (1,2). Aberrant expression of sugars on the surface of the cell is hallmark of diseases such as autoimmune disorders and neoplastic transformation (1). The discovery of lectins, a class of multivalent proteins that bind sugars selectively much as antibodies bind to antigens was a great step in the elucidation of the mechanism of cellular recognition in general and the role of sugars therein in particular. Because of their sugar selectivity lectins display blood group and tumor cell specific agglutination, mitogenicity etc. (2). Consequently they are used routinely in clinics and blood banks as well as in the isolation and purification of glycoproteins (3). As all the activities of lectins are manifestation of their sugar specificities and as they provide prototype model systems for the interactions that occur at cell surfaces as well as protein-sugar interactions, the combining sites of lectins have become objects of intense scientific scrutiny in our laboratory for the last decade. Thermodynamic investigations reported here pertain to the general features of protein-carbohydrate recognition and more specific ones of Winged bean (Psophocarpus tetragonolobus) basic agglutinin (WBAI) which has been shown by us earlier to interact exquisitely with blood group A-reactive sugars (4,5). These studies underscore the important part played by sugars not involved directly in binding in favourably orienting the interacting regions of sugars and the role of solvent (water) re-organization in these interactions.

## MATERIALS AND METHODS

Materials : Galactose (Gal), N-acetylgalactosamine (GalNAc), Galα6Gal and lactose were from Sigma, USA. Galα3Gal, Galα3GalαMe, Galβ3GalβMe, Galα4Gal, Galα4GalβEt, GalNAcβ3GalαMe were products of Carbohydrates International, Sweden. Blood group reactive sugars were purchased from Biocarb Chemicals, Sweden. GalNAcα3Gal (A-disaccharide) was prepared from GalNAcα(Fucα2)3Gal (A-trisaccharide) as described in Mahanta et al. (6). N-Dansyl galactosamine (GalNDns) and WBAI were prepared according to Khan et al. (4).

Thermodynamics of ligand binding to WBAI : The association constants ($K_a$) for various sugars were determined by substitution titration using GalNDns as the indicator ligand as described in Khan et al. (4) and Swamy et al. (9).The changes in enthalpies for the binding of sugars were evaluated from the temperature dependence of their Ka values using van't Hoff plots. Changes in entropies were then obtained by substituting $\Delta G$ and $\Delta H$ in the equation; $\Delta G = \Delta H - T\Delta S$

Molecular modelling : Molecular models for sugars were obtained using the Hard Sphere Exoanomeric Affect algorithm (7).

Determination of concentrations : Concentrations of WBAI, GalNDns and other sugars were determined according to Lowry's method (8), molar absorptivity of 4800 $cm^{-1}$ at 330 nm (4) and weights, respectively.

## RESULTS AND DISCUSSION

In a previous study we had shown that WBAI recognizes sugars in D-galacto configuration and prefers strongly GalαMe and GalNAcαMe over their β-linked counterparts (4). Mellibiose (Galα6Glc) bound better than lactose (Galβ4Glc). Immunochemical analyses of its interaction with blood group substances revealed that its specificity is directed towards blood group A and B antigens (5). These investigations reporting the binding of a variety of galactose and N-acetylgalactosamine containing oligosaccharides not only confirm these findings but also provide considerable informations about the ligand specificity of WBAI, topography of its combining site and the mechanism of its interaction with sugars.

Data in Table 1 show that the α-linked disaccharides of Gal and GalNAc are considerably better ligands than their β-linked counterparts, Galα3GalαMe, for example, is 95 times more potent as compared to Galβ3GalβMe. Since the primary specificity of WBAI is

**TABLE 1. Values of association constant $K_a$ and $-\Delta G$ for the binding of sugars to WBAI at 20°C**

| Ligands | $10^{-3} \times K_a$ ($M^{-1}$) | $-\Delta G$ $kJmol^{-1}$ | Relative affinity (galactose = 1) |
|---|---|---|---|
| Gal | 1.05 | 16.94 | 1.00 |
| L-Fuc | 0.18 | 12.65 | 0.17 |
| GalNAcα3Gal | 10.71 | 22.60 | 10.20 |
| GalNAcβ3GalαMe | 0.22 | 13.13 | 0.21 |
| Galα3Gal | 4.52 | 20.50 | 4.30 |
| Galα3GalαMe | 7.54 | 21.75 | 7.18 |
| Galβ3GalβMe | 0.08 | 10.67 | 0.076 |
| L-Fucα2Gal | 0.66 | 15.81 | 0.63 |
| Galα4GalβEt | 1.93 | 18.43 | 1.84 |
| Galα4Gal | 1.50 | 17.82 | 1.43 |
| Galβ4Glc | 0.04 | 8.99 | 0.038 |
| Galα6Glc | 2.19 | 18.74 | 2.16 |

**GalNAcα3Gal**
[A-disaccharide (A-Di)]

**GalNAcα3Galβ4Glc**
[A-trisaccharide (A-Tri)]

**GalNAcα(L-Fucα2)3Gal**
[Fucosylated A-trisaccharide (A-TriF)]

**GalNAcα(L-Fucα2)3Galβ4Glc**
[A-tetrasaccharide (A-Tetra)]

**GalNAcα(L-Fucα2)3Galβ(L-Fucα3)4Glc**
[A-pentasaccharide (A-Penta)]

**GalNAcα(L-Fucα2)3Galβ3GlcNAcβ3Galβ4Glc**
[A-hexasaccharide (A-Hexa)]

**GalNAcα(L-Fucα2)3Galβ(L-Fucα4)3GlcNAcβ3Galβ4Glc**
[A-heptasaccharide (A-Hepta)]

**L-Fucα2Galβ(L-Fucα3)4Glc**
[Difucosyllactose (DiFucL)]

**Fig.1. Structures of blood group A-reactive saccharides.**

directed towards galactose, it must occupy the primary subsite and as L-Fucα2Gal binds reasonably well this subsite must be complementary to the subterminal galactose residue. As L-fucose by itself is inactive WBAI must be able to bind the reducing end galactose residue in L-Fucα2Gal. These data also show that fucosylation of galactose residue in α1-2 linkage neither impairs the binding of the galactosyl residue appreciably nor enhances its affinity. It may be noted that amongst disaccharides GalNAcα3Gal is the best ligand followed by Galα3Gal which, respectively, are the blood group A and B reactive disaccharides. This indicates that the acetamido group at C-2 position of subterminal galactose structure makes a favourable contribution to the binding affinity which amounts to 2.5 $kJmol^{-1}$. Galα4Gal is 3 times poorer a ligand as compared to Galα3Gal. This is probably due to the close vicinity of the non-reducing end galactose to the reducing one which shields a substantial portion of the groups of the latter diminishing the affinity of Galα4Gal relative to Galα3Gal, as shown in (6), excepting that in contrast to Jacalin, WBAI binds to the reducing Gal.

**TABLE 2. Association constants (at 20°C) and thermodynamic parameters for the binding of WBAI to blood group A antigenic sugars.**

| Ligands | $10^{-3}XK_a$ ($M^{-1}$) | Relative affinity (GalNAc = 1) | $-\Delta G$ $kJmol^{-1}$ | $-\Delta H$ $kJmol^{-1}$ | $-\Delta S$ $Jmol^{-1}K^{-1}$ |
|---|---|---|---|---|---|
| GalNAc | 5.26 | 1.00 | 20.87 | 27.19 | 21.54 |
| A-Di | 10.71 | 2.04 | 22.60 | 34.54 | 40.73 |
| A-TriF | 12.41 | 2.36 | 22.96 | 34.26 | 38.58 |
| A-Tri | 94.14 | 17.90 | 27.90 | 44.00 | 54.95 |
| A-Tetra | 89.50 | 17.02 | 27.78 | 31.24 | 11.79 |
| A-Penta | 98.50 | 18.73 | 28.01 | 29.84 | 6.20 |
| A-Hexa | 124.24 | 23.62 | 28.57 | 24.59 | -13.59 |
| A-Hepta | 5.36 | 1.02 | 20.92 | 19.47 | -4.85 |
| DiFucL | No Binding | | | | |

Table 2 lists the association constants and thermodynamic parameters of blood group A reactive saccharides together with their relative affinities with GalNAc as the reference (Refer Fig. 1 for structures). Incorporation of a GalNAc residue in $\alpha$1-3 linkage to Gal as in GalNAc$\alpha$3Gal, increases the affinity 2.04 times. Addition of a Glc residue in $\beta$1-4 linkage to Gal of this disaccharide increases the binding propensity of the resultant trisaccharide, GalNAc$\alpha$3Gal$\beta$4Glc by eight fold. A-trisaccharide is thus 17.90 and 8.8 times more potent a ligand over GalNAc and A-Di (Fig.1), respectively. However the presence of a Fuc in $\alpha$1-2 linkage to Gal residue of A-Di as in GalNAc$\alpha$(Fuc$\alpha$2)3Gal increases the affinity only by 1.16 over the corresponding A-Di. Increase in the enthalpies for the binding of A-Tri over that observed for A-Di suggests that the lectin has an extended combining site complementary to all the constituent monosaccharides of A-Tri.

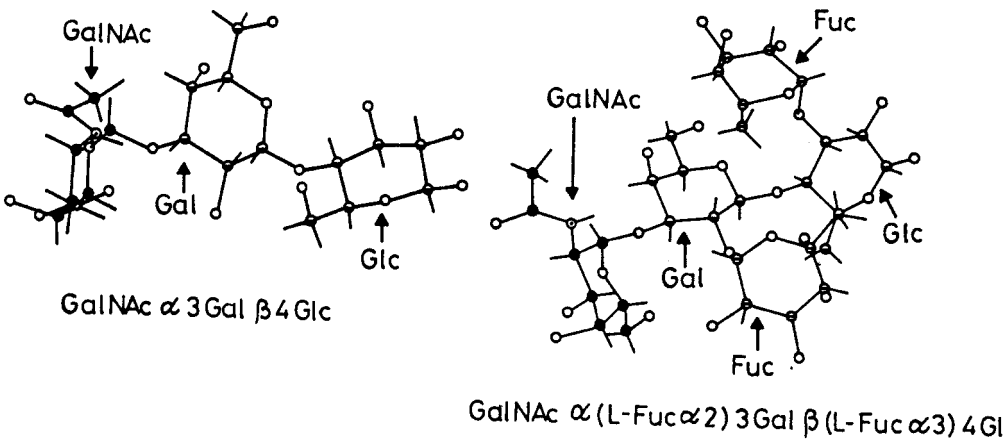


**Fig.2. Pluto plots of the preferred orientation of sugar residues in non-fucosylated A-Tri [GalNAc$\alpha$3Gal$\beta$4Glc] and the difucosylated A-Penta [GalNAc$\alpha$(L-Fuc$\alpha$)3Gal$\beta$(L-Fuc$\alpha$3)4Glc].** The carbon atoms of the terminal GalNAc are shown by filled circles (●), subterminal Gal and Glc by half filled circles (⊖) and those of fucoses by circles with a bar (⊖). The combining site of WBAI is complementary to the linear non-fucosylated trisaccharide. Terminal GalNAc is indispensable and it occupies the deep groove of the combining site. However, subterminal Gal and Glc resides are in the shallow region of the binding site. Both fucoses of the A-Penta project away from the binding site. Note that upon fucosylation the structure of the trisaccharide shown left becomes more favourable for the interaction to take place as shown in the right side of the figure. O—O, oxygen atoms; ⊙—⊙, nitrogen atoms. Hydrogen atoms have been omitted.

Non-fucosylated A-Tri [GalNAc$\alpha$3Gal$\beta$4Glc], monofucosylated A-Tetra [GalNAc$\alpha$(Fuc$\alpha$2)3Gal$\beta$4Glc], and difucosylated A-Penta [GalNAc$\alpha$(Fuc$\alpha$2)3Gal$\beta$(Fuc$\alpha$3)4Glc] have very similar free-energy values, which would mean that both of the fucose residues of this pentasaccharides do not make any direct contribution to the binding energy of the ligands to WBAI. This is also evident from the fact that addition of fucose residues does not change enthalpy. Since difucosyllactose, which lacks the terminal GalNAc of the A-Penta is inactive, the terminal $\alpha$1-3 linked GalNAc of blood group A reactive pentasaccharide must make a major contribution to the binding energy. Hence the terminal $\alpha$1-3 linked GalNAc must be buried in a deep cleft in the combining site of the lectin. The subterminal Gal and Glc residues of A-Tri

(GalNAcα3Galβ4Glc) make marginal contribution to the binding energies. Interestingly enough, despite the non-interaction of the fucosyl residues of A-Penta with the lectin they entropically favour the binding of oligosaccharide by suitably orienting the binding epitope (GalNAcα3Galβ4Glc) of the saccharide.

A-Hexa [GalNAcα(Fucα2)3Galβ3GlcNAcβ3Galβ4Glc], which has β1-3 linked GlcNAcβ3Galβ4Glc to the Gal is about 1.4 times better ligand than A-Tetra, which would imply that the replacement of β4Glc of A-Penta with β3GlcNAc favours the binding of the A-Hexa. Alternatively additional residues make some favourable contacts with the protein. Addition of a fucose residue in α1-4 linkage at β3GlcNAcβ of A-Hexa giving A-Hepta [GalNAcα(L-Fucα2)3Galβ(L-Fucα4)3GlcNAc 3Galβ4Glc] reduces the affinity of the latter by 23.18 times. Thus the presence of a fucose residue in α1-4 linkage highly destabilizes the protein-ligand complex, which may be due to steric reasons. Due to this steric hinderance A-Hepta presumably interacts with its terminal GalNAc alone which is also attested to by its affinity which is close to that of GalNAc itself. Fucose in α1-4 linkage in A-Hepta may also impart an unfavourable orientation to the binding epitope in contrast to the α1-2 and α1-3 linked fucose residues of A-pentasaccharide allowing the interaction of terminal GalNAc alone.

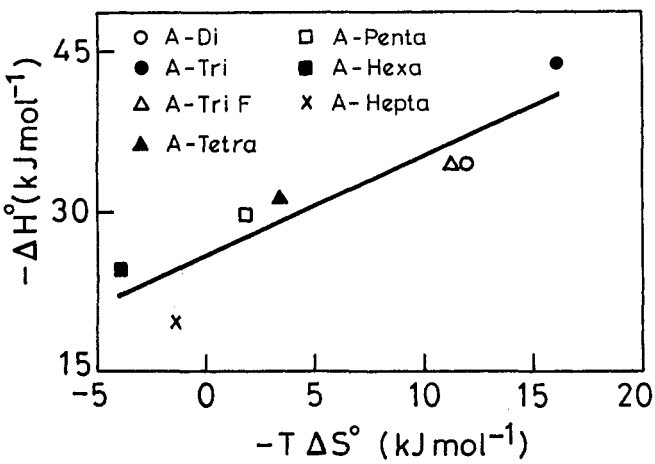


**Fig.3. A plot of $-\Delta H^{\circ}$ versus $-T\Delta S^{\circ}$ at 20°C for blood group A reactive sugars.** The straight line is the least squares fit of the data to a straight line.

An examination of the thermodynamic parameters for blood group A-reactive sugars also reveals an enthalpy-entropy compensation for the binding process, indicating that these interactions which are primarily enthalpically driven are accompanied by reorganization of water around both the lectin and carbohydrate. Such enthalpy-entropy compensation have also been observed by us in many other lectin-sugar interactions (11). Precedences for water being the predominant source determining the thermodynamics of protein-ligand interactions are also speculated in other systems (12-14).

Failure to observe appreciable conformational rearrangement(s) for either protein or sugar as well as the complex (4,9,11) together with the linearity of van't Hoff plots for each of the sugar examined indicates that these interactions are accompanied by little heat capacity changes which would give credence to our suggestion that reorganization of water is the major driving force for the lectin-carbohydrate recognition.

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