A predominantly hydrophobic recognition of H-antigenic sugars by winged bean acidic lectin: a thermodynamic study

V.R. Srinivas, G. Bhanuprakash Reddy, Avadhesa Surolia*

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India

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Abstract The thermodynamics of binding of winged bean (*Psophocarpus tetragonolobus*) acidic agglutinin to the H-antigenic oligosaccharide (Fucα1-2Galβ1-4GlcNAc-oMe) and its deoxy and methoxy congeners were determined by isothermal titration calorimetry. We report a relatively hydrophobically driven binding of winged bean acidic agglutinin to the congeners of the above sugar. This conclusion is arrived, from the binding parameters of the fucosyl congeners, the nature of the enthalpy-entropy compensation plots and the temperature dependence of binding enthalpies of some of the congeners. Thus, the binding site of winged bean acidic agglutinin must be quite extended to accommodate the trisaccharide, with non-polar loci that recognize the fucosyl moiety of the H-antigenic determinant.

Key words: Scanning calorimetry; Congener; Hydrophobicity

1. Introduction

Lectins are a class of multivalent carbohydrate binding proteins with a high degree of specificity towards their respective sugar ligands. The ability of lectins to discriminate subtle variations in glycoconjugate structures has many applications, as highly discriminating probes in the separation of normal and neoplastic cells, the study of cell surface architecture during development processes, tissue and blood typing etc. Hence, the nature and mechanisms of binding of lectins to their sugar ligands have been the subject of intense investigations [1].

The interaction of proteins to their respective ligands, in general, is an outcome of a network of forces that make such interactions remarkably specific, with a high degree of affinity, a hallmark of biological recognition. While for most protein-ligand systems, hydrogen bonding and van der Waal's forces are thought to impart specificity to the interactions, hydrophobic interactions are believed to constitute the major driving force for the process [2]. On the other hand, in the case of lectin-sugar binding, hydrogen bonding is believed to be the predominant force, because of the participation of the numerous hydroxyl groups of the sugars [3]. Typically in legume lectins, one of the best studied sugar-protein interaction systems, there are 5–9 hydrogen bonds between the monosaccharide and protein residues, either directly or through water molecules [4–7]. Another characteristic feature of many protein-ligand interactions, including that of lectin-carbohydrate binding, is the compensatory nature of enthalpy and entropy changes, an observation usually interpreted as due to release or uptake of water upon binding, which emphasizes the importance of water as a mediator in these recognition processes [4–12]. Also, many protein-sugar interactions exhibit relatively insignificant changes in heat capacity upon ligand binding. Where such heat capacity changes are observed, which are usually negative in nature, they constitute compelling evidence for the role of hydrophobic interactions as the driving force for the association [13–15].

In the present investigation, we report an isothermal titration calorimetric (ITC) study of the binding of winged bean (*Psophocarpus tetragonolobus*) acidic agglutinin (WBA II) to H-type II-oMe sugar (Fucα1-2Galβ1-4GlcNAc-oMe) and a series of its deoxy and methoxy congeners. This lectin, which is isolated from the seeds of the legume winged bean, is a homodimeric glycoprotein with an isoelectric point of 5.5 and Mr = 54 000. WBA II binds specifically to the terminal fucosylated H-antigenic determinant either on human erythrocytes or in solutions [16,17]. In the present study, we report the relative contributions of various hydroxyl groups of H-type-II-oMe to hydrogen bonding with the lectin and, in addition, note a change in the heat capacity upon ligand binding. Such heat capacity changes upon sugar binding have not been observed for any lectin-sugar interaction and provides strong evidence for the relatively greater contribution of non-polar forces in the recognition of WBA II to its fucosylated ligands.

2. Materials and methods

2.1. Materials

The H-type-II-oMe sugars and its deoxy and methoxy analogues were generous gifts of Dr Ulrike Spohr and Prof. R.U. Lemieux of the University of Alberta (Edmonton, Alta, Canada). WBA II was prepared and purified to a purity higher than 98%, as previously described [16,17]. Sodium dodecylsulfate polyacrylamide gel electrophoresis showed only one band with a relative molecular mass, M_r of 27 000, for one subunit. De-ionized and double-distilled water was used for all the solutions. The sodium phosphate, phosphoric acid and sodium chloride were obtained from Merck (Bombay, India). Sodium phosphate and sodium chloride were purified by recrystallization.

2.2. Preparation and analysis of solutions

The WBA II solutions were prepared in 0.15 M sodium chloride, 0.02 M sodium phosphate buffer at pH 7.4 (PBS), dialyzed overnight in a large volume of the same buffer and centrifuged to remove any insoluble material. The concentration of the protein solutions was determined by using the lectin specific absorbance $A^{1\%}_{280\,nm} \sim 7.7$.

Solutions of carbohydrates were prepared by weight in the dialysate to minimize differences between the protein buffer solution and ligand buffer solution in the ITC measurements.

2.3. ITC measurements and analysis

The ITC measurements were performed with a Microcal Omega titration calorimeter as described previously [18–20]. Aliquots of the

*Corresponding author. Fax: (91) (80) 3348535.
E-mail: surolia@mbu.iisc.ernet.in

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ligand solution at 10–20× the binding site concentration were added via a 250 μl rotating stirrer syringe to the solution cell containing 1.34 ml of the 0.18 mM–0.20 mM protein solution. The heats of dilution was determined to be negligible in separate titrations of the ligand solution into the buffer solution. An identical two-site model, utilizing a site concentration = 2×[WBA II], P_t, was the simplest binding model found to provide the best fit with the ITC data. The total heat content, Q_t, is related to the total ligand concentration, L_t, according to the following equation:

$$Q_t = 2nP_t\Delta H_b V(1 + L_t/2nP_t + 1/2nK_bP_t -$$

$$((1 + L_t/2nP_t + 1/2nK_bP_t)^2 - 4L_t/2nP_t)^{1/2})/2 \quad (1)$$

where n is the stoichiometry and V is the cell volume. The expression for the heat released per the i^{th} injection, $\Delta Q_{(i)}$, is then [18,19]

$$\Delta Q_{(i)} = Q_{(i)} + dV_i/2V(Q_{(i)} + Q_{(i-1)}) - Q_{(i-1)} \quad (2)$$

where dV_i is the volume of the titrant added to the solution. Fits of this identical two-site model to the titration data yielded values for n, ΔH_b and K_b, the site binding constant.

The thermodynamics quantities, ΔG_b^0 and ΔS_b, were obtained from

$$\Delta G_b^0 = \Delta H_b - T\Delta S_b \quad (3a)$$

where

$$\Delta G_b^0 = -nRT\ln(K_b) \quad (3b)$$

and n is the number of moles, T is the absolute temperature in K and $R = 0.00198$ kcal/mol/K.

The van 't Hoff enthalpies are calculated from the equation

$$\ln(K_{bT}/K_{bTo}) = -\Delta H_{bv}(1/T_o - 1/T)/R \quad (4)$$

$$\Delta H_{bT} = \Delta H_{b0} - \Delta C_p(T - T) \quad (5)$$

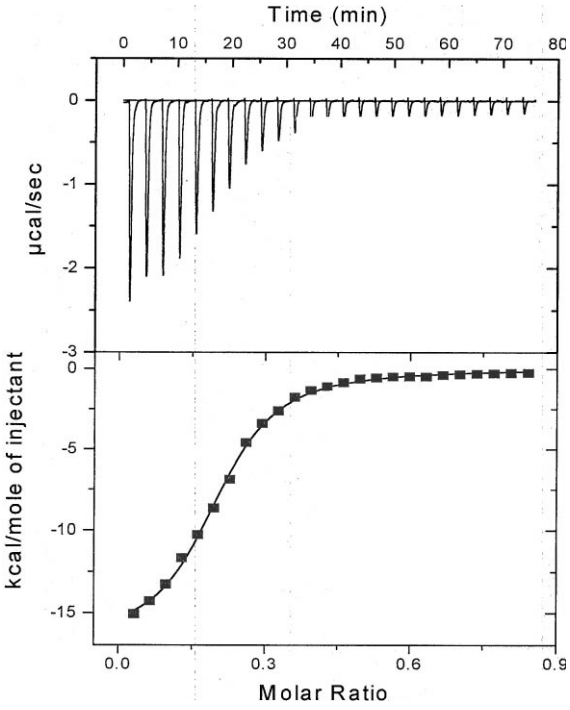

Fig. 1. The results of a typical ITC experiment, which consisted of adding 5.8 μl of 1.4 mM H-type-II-oMe sugar to 1.34 ml of 0.18 mM (monomer) WBA II in PBS, pH 7.4, at 298 K

3. Results and discussion

In this paper, we report an ITC study of the binding of WBA II to Fucα1-2Galβ1-4GlcNAc-oMe (Fig. 1) and a series of its deoxy and oMe congeners. The role of specific OH groups in their interactions with a protein can be studied by comparing the binding of the deoxy congeners and the parent ligand to the protein. In addition, the contribution of hydrophobic interactions to the binding process can be determined by replacement of the OH by an apolar substituent, such as an oMe group [21]. Earlier, our group reported the thermodynamics of binding of some deoxy congeners, by their ability to inhibit binding of the fluorescent reporter ligand, *N*-dansylgalactosylamine [22]. The binding constants were calculated by competition between the fluorescent and the deoxy sugars for interaction with the lectin. The binding enthalpies were determined from the van 't Hoff equation (Eq. 4) where one assumes a temperature independence of the binding enthalpies, an assumption that may not apply for all reactions. On the other hand, ITC offers the advantage of direct measurement of enthalpy changes, in addition to providing an accurate estimate of both the stoichiometry and the binding

Table 1
Thermodynamic binding parameters of H-type-II-oMe sugar and its analogues to WBA II

Ligand	T (T)	N	K_b	$-\Delta H_b$ (kcal/mol)	$-\Delta G_b$ (kcal/mol)	ΔS_b (cal/mol/K)	Relative affinity
H-type-II-oMe	283.1	0.86	3.068×10^5	13.70	7.08	−23.4	1.0000
3a-deoxy	283.0	0.84	2.07×10^5	10.54	6.86	−13.00	0.6747
3a-oMe	283.0	0.95	5.57×10^4	6.080	6.12	0.1520	0.1816
6a-deoxy	283.0	0.84	2.73×10^5	11.04	7.01	−14.20	0.8898
6a-pavalamido	283.1	0.81	4.93×10^5	16.81	7.34	−33.40	1.6069
3b-deoxy	282.8		NB				
3b-oMe	283.0		NB				
4b-deoxy	283.0		NB				
4b-oMe	283.0		NB				
6b-deoxy	283.1	0.88	3.53×10^4	6.180	5.87	1.090	0.1151
6b-oMe	283.1	0.94	9.9×10^4	10.60	6.45	−14.7	0.3227
2c-deoxy	282.9	0.80	6.2×10^4	10.70	6.18	−16.0	0.2021
2c-oMe	283.0		NB				
3c-deoxy	283.0	0.85	3.16×10^5	7.650	7.09	1.960	1.0300
3c-oMe	283.0	0.71	7.79×10^3	1.780	5.02	11.5	0.0254
4c-deoxy	283.0	0.80	3.6×10^5	12.12	7.16	−17.5	1.1734
4c-oMe	283.0	0.73	6.3×10^5	11.60	7.48	−14.5	2.0535
6c-nor	282.9	0.97	1.33×10^5	9.150	6.61	9.00	0.4335

The relative affinity is calculated with respect to H-type-II-oMe as the ratio of K_b of sugar to K_b of H-type-II-oMe. Each value is an average of 2–3 determinations.

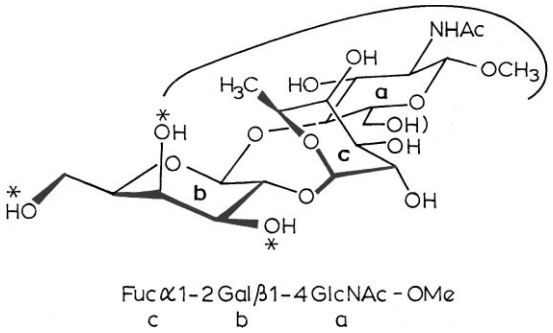

Fig. 2. The structure of H-type-II-oMe sugar. The asterisks show the hydroxyl groups that may be involved in direct hydrogen bonding with WBA II while the continuous line depicts the surface of the saccharide molecule being recognized hydrophobically.

constant in a single experiment. Moreover, the calorimetric enthalpy of binding may be evaluated at different temperatures and its change as a function of temperature, and hence the consequent change in the heat capacity during the reaction can be estimated (Eq. 5). Measuring changes in heat capacity provides important insights into the energetics and mechanism of binding of ligands to protein. In the present study, we observe that while we get higher values for enthalpies, the changes in free energy of binding are relatively similar to that obtained from the earlier fluorescent studies. Such a variance between the calorimetric binding enthalpy and the indirectly obtained binding enthalpy (from fluorescence studies) has been reported earlier [23–25]. In addition, in the present study, we present data on the binding of oMe analogues and provide evidence for the effect of temperature on the enthalpy of binding, that further delineates the mechanism of recognition of the H-antigenic sugars to WBA II.

Our data (Table 1) establish that WBA II binds to H-type-oMe sugar, with a marginally better association constant (K_a) and binding enthalpy (ΔH_b) as compared to fucosyllactose, suggesting that the acetamido group of the former contributes appreciably to the binding process. An 85-fold stronger affinity of the H-type-oMe determinant over 2-fucosylgalactose (H-disaccharide) implies an extended binding site for WBA II where the reducing end, α-methyl-2-deoxy-*N*-acetylglucosamine, is accommodated [26]. Moreover, 3b-, 4b-, 6b- and 2c-deoxy and oMe analogues show a very reduced binding to WBA II, confirming earlier studies that the hydroxyls at position 3, 4 and 6 of the galactosyl moiety and the two hydroxyls of the fucosyl moiety may provide the primary hydrogen bonding interactions in its binding to WBA II. Interestingly, while the 3a-deoxy analogue showed about a 30% reduction in its binding to WBA II, its oMe counterpart shows a 10-fold lower binding then the parent ligand, H-II-type-oMe, suggesting that the bulky oMe group, at C-3 of its reducing end, GlcNAc, sterically hinders the binding of the sugar to the protein. Unusually, the 6a-pivalamido analogue which binds with a higher affinity then the parent molecule is nevertheless enthalpically driven, despite the fairly bulky pivalamido group replacing the OH at position 6 of the GlcNAc moiety. The structure of the pivalamido group is $(CH_3)CCONH_2$. Perhaps, hydrogen bonding between the amide group and a protein residue may be the major contributing factor in the binding reaction. This point may be noted in the light of the binding parameters for the 6a-deoxy analogue. The 6a-deoxy congener shows only a marginal (11%) reduction in its binding as compared to H-type-II-oMe. Perhaps, a very weak hydrogen bonding may be 'hooking' this group to a fairly distant residue on the binding site of the lectin. The bulkier pivalamido group of the congener, perhaps, bridges this distance and reinforces the hydrogen bonding reaction through its amido group.

The fucosyl moiety of the trisaccharide shows some interesting features, as revealed by the binding parameters of the fucosyl congeners. The binding parameters show the importance of the 2c-OH group in its interaction with WBA II. Both the deoxy and the methoxy analogues show a highly reduced affinity for WBA II. In fact, while the 2c-deoxy analogue shows a 10-fold decrease in its binding, the methoxy congener shows no binding at all. On the other hand, while the binding of the 4c-deoxy analogue is marginally higher, the 4c-methoxy analogue binds much stronger than the H-type-II-oMe sugar. Interestingly, this binding has a lower enthalpy change than the parent molecule and hence has a greater entropic contribution to the binding process. Similarly, the binding of the 6c-nor analogue is very highly entropic in nature. It hence seems, in the light of the above discussion, that the WBA II binding site for the fucosyl moiety, distinct from the galactose binding site, indeed has a considerable hydrophobic character. These data also show that the hydroxyl groups at C3, C4 and C6 of the β-galactosyl moiety and the C2 hydroxyl of the α-fucosyl residue are involved in hydrogen bonding to the corresponding loci in WBA II, whereas the rest of the molecule interacts predominantly through hydrophobic interactions (Fig. 2).

Typical of many protein-ligand interactions, especially that of lectin-carbohydrate interactions, is the compensatory behavior of the enthalpy and entropy changes upon binding [4–12]. This observation is usually interpreted as due to release and uptake of water upon binding of the ligand. Removal of water from the interacting surface contributes significantly to the binding energy, ΔG_b^0, due to the favorable entropic nature of the process. On the other hand, a net inclusion of water

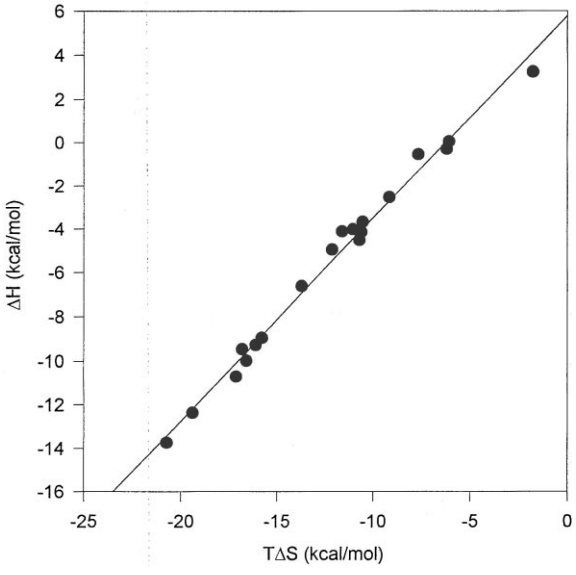

Fig. 3. Enthalpy-entropy compensation plot of WBA II-sugar interactions. The slope of this plot is 0.90 and the correlation coefficient is 0.98.

Table 2
The effect of temperature on the binding parameters. ΔC_p is calculated from Eq. 5

Ligand	T (K)	N	K_b	$-\Delta H_b$ (kcal/mol)	$-\Delta G_b$ (kcal/mol)	ΔS_b (cal/mol/K)	Relative affinity
H-type-II-oMe	283.1	0.86	3.068×10^5	13.70	7.08	−23.4	1.0
	298.0	0.94	1.07×10^5	16.10	6.83	−31.4	0.3488
	308.0	0.96	4.4×10^4	17.12	6.41	−35.3	0.1434
					$\Delta C_p = -0.14$ kcal/K/mol		
6a-pavalamido	283.1	0.81	4.93×10^5	16.81	7.34	−33.4	1.6069
	293.0	0.81	1.7×10^5	20.73	6.98	−46.9	0.5541
					$\Delta C_p = -0.39$ kcal/K/mol		
4c-deoxy	282.9	0.79	3.6×10^5	12.12	7.16	−17.5	1.1734
	298.0	0.83	8.86×10^4	16.59	6.61	−34.1	0.2888
					$\Delta C_p - = 0.29$ kcal/K/mol		
4c-oMe	283.0	0.73	6.3×10^5	11.60	7.48	−14.5	2.0535
	298.0	0.88	1.31×10^5	15.78	6.83	−30.5	0.4270
	308.0	084	1.24×10^5	19.40	7.03	−40.8	0.4042
					$\Delta C_p = -0.31$ kcal/K/mol		

Each value is an average of 2–3 determinations.

during binding should be unfavorable to the binding reaction. In the latter case, the occurrence of binding implies that the loss in entropy, due to the ordering of water molecules, should be compensated by a favorable binding enthalpy. In fact, a typical enthalpy-entropy compensation plot may be taken as indirect evidence of the involvement of solvent in the binding reaction. Studies on several lectins strongly emphasize the importance of water as a mediator of protein-carbohydrate recognition and recently, we have provided direct evidence for the involvement of water in the binding of concanavalin A to mannooligosaccharides [27]. An exact compensation of enthalpy by entropy has a slope close to one [24]. On the other hand, deviation from this value suggests different energetic mechanisms to the binding process [8,10–12]. A ΔH_b versus $T\Delta S_b$ plot with a slope greater than one suggests that the process is predominantly enthalpy driven [10]. On the other hand, a plot with a slope less than one is suggestive of a relative predominance of the entropic factor in binding [11,12]. The thermodynamics of binding of WBA II to H-type-II-oMe and its congeners confirm the notion of the involvement of water in the binding reaction since the enthalpy changes are linearly compensated by the entropy of binding (Fig. 3). But the slope of the plot is 0.9, suggesting that the binding process is relatively more favored by a change in the entropy. This indeed seems to confirm that the energetics of binding of WBA II to fucosylated saccharides is relatively more hydrophobically driven.

Another interesting facet of the present investigation is the observation of the distinct change in enthalpy with temperature, and a consequent change in the heat capacity upon binding (Table 2). Such an observation has not yet been noted for any lectin-sugar interaction. We observe a distinct change in the heat capacity (−150–−300 kcal/mol/K) upon binding of a H-type-II-oMe sugar and some of its analogues, further confirming the interpretation that the binding to the fucosylated sugars has a distinct hydrophobic character.

Thus, binding of WBA II to fucosylated sugars is accompanied by moderately significant changes in heat capacities. This, in addition to the compensatory nature of enthalpy and entropy changes with a slope less than one, particularly with respect to the fucosyl congeners, is strongly suggestive of a hydrophobically driven binding process with the removal of water from the binding site, probably in the vicinity of fucose, contributing significantly to the binding reaction. This in turn would suggest that the fucosyl moiety abuts close to predominantly non-polar side chains in the combining site of the lectin. Determination of the amino acid sequence of WBA II and its crystal structure complexed to the H-antigenic sugar, currently in progress, would serve to identify the hydrophobic binding site that contributes to this unique property of this lectin.

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