Effect of substituent on the thermodynamics of D-glucopyranoside binding to concanavalin A, pea (*Pisum sativum*) lectin and lentil (*Lens culinaris*) lectin

Frederick P. SCHWARZ[†], Sandra MISQUITH[‡] and Avadhesha SUROLIA[‡]

*Center for Advanced Research in Biotechnology/National Institute of Standards and Technology, 9600 Gudelsky Drive, Rockville, MD 20850, U.S.A., and [‡]Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

Titration calorimetry measurements of the binding of phenyl- α (α PhOGlu), 3-methoxy (3MeOGlu), fluorodeoxy and deoxy derivatives of α -D-glucopyranose (Glu) to concanavalin A (conA), pea lectin and lentil lectin were performed at approx. 10 and 25 °C in 0.01 M dimethylglutaric acid/NaOH buffer, pH 6.9, containing 0.15 M NaCl and Mn^{2+} and Ca^{2+} ions. Apparently the 3-deoxy, 4-deoxy and 6-deoxy as well as the 4-fluorodeoxy and 6-fluorodeoxy derivatives of Glu do not bind to the lectins because no heat release was observed on the addition of aliquots of solutions of these derivatives to the lectin solutions. The binding enthalpies, ΔH^0_b , and entropies, ΔS^0_b , determined from the measurements were compared with the same thermodynamic binding parameters for Glu, D-mannopyranoside and methyl- α -D-glucopyranoside (α MeOGlu). The binding reactions are enthalpically driven with little change in the heat capacity on binding, and exhibit enthalpy–entropy compensation. Differences between the thermodynamic binding parameters can be rationalized in

terms of the interactions apparent in the known crystal structures of the methyl- α -D-mannopyranoside-conA [Derewenda, Yariv, Helliwell, Kalb (Gilboa), Dodson, Papiz, Wan and Campbell (1989) EMBO J. 8, 2189–2193] and pea lectin-trimannopyranoside [Rini, Hardman, Einspahr, Suddath and Carber (1993) J. Biol. Chem. 268, 10126–10132] complexes. Increases in the entropy change on binding are observed for α MeOGlu binding to pea and lentil lectin, for α PhOGlu binding to conA and pea lectin, and for 3MeOGlu binding to pea lectin relative to the entropy change for Glu binding, and imply that the phenoxy and methoxy substituents provide additional hydrophobic interactions in the complex. Increases in the binding enthalpy relative to that of Glu are observed for deoxy and fluoro derivatives in the C-1 and C-2 positions and imply that these substituents weaken the interaction with the surrounding water, thereby strengthening the interaction with the binding site.

INTRODUCTION

The high specificity of carbohydrate binding to lectins makes this binding interaction an excellent model for investigating the effect of ligand structure on the binding thermodynamics of ligands to proteins. Information from this type of investigation would be useful in correlating thermodynamic binding parameters with interactions at the molecular level between the ligand and the amino acid residues at the protein binding site. Previous investigations in elucidating the effects of carbohydrate substituents on the binding thermodynamics to lectins have focused on monitoring changes in the Gibbs energy, commonly referred to as the binding affinity, ΔG^0_b , of carbohydrate derivatives binding to concanavalin A (conA) [1–4]. ΔG^0_b is, however, a function of changes in the enthalpy (ΔH^0_b) and entropy (ΔS^0_b) parameters of the binding interaction through the fundamental equation of thermodynamics:

$$\Delta G^0_b = \Delta H^0_b - T\Delta S^0_b \quad (1)$$

where T is the temperature in K. Enthalpy–entropy compensation, where changes in the binding enthalpy are compensated for by changes in the binding entropy, has been observed for many lectin–carbohydrate complexes [5], which further obscures the effects of changes in the intermolecular interactions on ΔG^0_b . To relate the thermodynamic parameters of the binding reaction specifically to interactions at the molecular level, it is important to determine changes in both ΔH^0_b and ΔS^0_b .

More recent studies [5–8] indicate that the interaction between the carbohydrate and lectins is predominately hydrogen-bonding between the OH groups on the carbohydrate and the amino acid residues at the binding site. Replacement of an OH group with H on the carbohydrate would eliminate the contribution of that group to the binding thermodynamics, whereas replacement with F would restrict the group contribution to that of an acceptor in hydrogen bond formation [9] with the amino acid residues of the lectin. Thus by monitoring changes in ΔG^0_b , ΔH^0_b and ΔS^0_b resulting from H or F substitution for an OH group on the carbohydrate, the contribution of that particular OH group to the binding thermodynamics can be assessed.

In the present study, titration calorimetry is used to determine the thermodynamic parameters, ΔH^0_b and ΔG^0_b , at 10 and 25 °C for the binding of deoxy- and fluoro-substituted D-glucopyranoside derivatives to conA, pea lectin and lentil lectin. More specifically, the deoxy derivatives consisted of replacement of the OH group with H at C-1 (1HGlu), C-2 (2HGlu), C-3 (3HGlu), C-4 (4HGlu) and C-6 (6HGlu) and the fluoro derivatives of replacement of OH with F yielding the corresponding substituted Glu derivatives 1FGlu, 2FGlu, 3FGlu, 4FGlu and 6FGlu. The resulting changes in the thermodynamic parameters were then compared with changes in the thermodynamic parameters for α Glu binding from an earlier study [5]. The structure of the methyl- α -D-mannopyranoside (α MeOMan)–conA complex determined by X-ray crystallography [10] was used for tentative identification of the hydrogen bonds between groups on the carbohydrate derivative and the amino acid residues at the

Abbreviations used: conA, concanavalin A; Glu, α -D-glucopyranose, α MeOGlu, methyl- α -D-glucopyranoside; DMG, dimethylglutaric acid/sodium hydroxide buffer; FGlu, fluoro-1-deoxy-D-glucopyranoside; HGlu, deoxy-D-glucopyranoside; 3MeOGlu, 3-O-methyl-D-glucopyranoside; α PhOGlu, phenyl- α -D-glucopyranoside.

[†] To whom correspondence should be addressed.

binding sites of conA. In addition, changes in the thermodynamic parameters for the binding to these lectins of the 'bulky' substituted phenyl- α -D-glucopyranoside (α PhOGlu) and 3-*O*-methyl- α -glucopyranoside (3MeOGlu) derivatives were determined and also compared with changes in the thermodynamic parameters for Glu binding. A comparison of the changes in the thermodynamic parameters, including those of methyl- α -D-glucopyranoside (α MeOGlu) from an earlier study [5], can elucidate contributions to the binding thermodynamics from hydrophobic interactions of the phenyl and methyl groups with the amino acid residues at the binding site.

EXPERIMENTAL

Materials

The Glu derivatives, except 1HGlu and 4HGlu, and lectins were obtained from Sigma Chemical Co. The pea and lentil lectins were also prepared as described previously [5]. The pea and lentil lectins obtained from Sigma exhibited the same binding thermodynamic parameters as those prepared at the Indian Institute of Science in Bangalore [5]. 1HGlu and 4HGlu were prepared in accordance with the procedure described by Street et al. [9]. Dimethylglutaric acid, sodium hydroxide, sodium chloride, manganese chloride and calcium chloride were obtained from the Sigma Chemical Co.

The 0.01 M dimethylglutaric acid/sodium hydroxide buffer containing 1 mM each of MnCl_2 and CaCl_2 at pH 6.85 ± 0.05 (DMG) was prepared by adding known masses of the solutes to distilled water. The pH was determined by an Orion pH meter. The lectin solutions were also prepared by weight, dialysed overnight, centrifuged and analysed by UV spectroscopy to determine the lectin concentration as described previously [5]. The Glu derivative solutions were prepared fresh by weight in the DMG dialysate from the lectin dialysis.

Titration calorimetry

The titration calorimetry measurements were made as described previously [5]. Briefly, 5 to 10 μl aliquots of the derivative solution were added to the lectin solution contained in a 1.385 ml cell and heat changes accompanying the additions were recorded. The total lectin concentration, $[\text{P}]_t$, was varied from 0.2 to 0.6 mM and the total concentration of derivative solution, $[\text{L}]_t$, was varied from 10 to 100 mM. The heats of dilution of the derivative solutions as determined from additions of the derivative solution to the buffer solution alone were negligible over this concentration range. The time scale between the additions was at least 3 min to allow the peak to return to the baseline, and the titrations were continued until the peak areas had decreased to one-half or less. The heat absorbed or released accompanying the addition of aliquots of the ligand solution to the protein solution is measured by a thermoelectric device between the solution and reference cells. As described by Yang [11], the heat content of the solution is related to the total ligand and total protein concentrations by:

$$Q_t = n[\text{P}]_t \Delta H^0_b V \left\{ 1 + [\text{L}]_t / n[\text{P}]_t + 1/nK_b[\text{P}]_t - [(1 + [\text{L}]_t / n[\text{P}]_t + 1/nK_b[\text{P}]_t)^2 - 4[\text{L}]_t / n[\text{P}]_t]^{1/2} / 2 \right\} \quad (2)$$

where n is the number of sites or stoichiometry, and V is the cell volume. For the heat released by the i th injection, $\Delta Q(i)$:

$$\Delta Q(i) = \Delta Q(i) + \delta V_i / 2V [\Delta Q(i) + Q(i-1)] - Q(i-1) \quad (3)$$

where δV_i is the volume of titrant added to the solution. Each titration calorimetry run yields ΔH^0_b and K_b from a least-squares fit of $\Delta Q(i)$ to eqns. (2) and (3). The stoichiometry of the fit was

fixed at $n = 1.00$ and $[\text{P}]_t$ = the total concentration of sites, which is twice the total protein concentration because the lectins are dimers with one site per each monomer subunit. Values for ΔS^0_b were obtained from eqn. (1). For $[\text{P}]_t$ = the concentration of sites, K_b is the same as the intrinsic site binding constant.

RESULTS

A typical titration curve of a derivative, 2FGlu, is shown in Figure 1 along with a fit of the incremental heats of binding to the injection number. The titration consisted of adding 10 μl aliquots of 30.4 mM 2FGlu to 0.379 mM pea lectin at 9.8 °C. As shown by the solid curve in Figure 1b, the incremental heats per mol of added ligand follow closely the injection number as determined by eqns. (2) and (3). The thermodynamic parameters ΔG^0_b , ΔH^0_b and ΔS^0_b at approx. 10 and 25 °C determined from the titration calorimetry measurements are presented in Table 1 for conA, Table 2 for pea lectin and in Table 3 for lentil lectin. Binding of 3HGlu, 4HGlu, 4FGlu, 6HGlu and 6FGlu to conA and pea and lentil lectins was not observed calorimetrically. The uncertainties in Tables 1–3 are standard deviations of the mean values of K_b , ΔG^0_b , ΔH^0_b and ΔS^0_b .

The binding enthalpies were also calculated from the following form of the van't Hoff equation:

$$\ln\{K_b\} = \Delta H^0_{bv} / RT + C \quad (4)$$

where $R = 8.315 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ and C is a constant. Values for ΔH^0_{bv} were calculated from the slope of the best fit of $\ln\{K_b\}$ to $1/T$. Because the fits were performed over a small temperature range, the standard deviation of the calculated van't Hoff enthalpies was higher than that of the calorimetric enthalpies. Agreement between the van't Hoff enthalpies (ΔH^0_{bv}) and the

Figure 1 Calorimetric titration of 2FGlu solution into pea lectin solution

(a) Titration of 10 μl aliquots of 30.4 mM 2FGlu in DMG with 0.379 mM pea lectin in DMG at 9.8 °C. (b) Fit of the incremental heats for the titration in (a) by injection number to eqns. (2) and (3) in the text.

Table 1 Thermodynamic binding quantities of glucopyranoside derivatives to concanavalin A

	Temp. (°C)	K_b (M ⁻¹)	$-\Delta G_b^0$ (kJ·mol ⁻¹)	$-\Delta H_b^0$ (kJ·mol ⁻¹)	$-T\Delta S_b^0$ (kJ·mol ⁻¹)	$-\Delta S_b^0$ (J·mol ⁻¹ ·K ⁻¹)
α PhOGlu	8.8	$1.20 \pm 0.14 \times 10^4$	22.0 ± 0.3	14.6 ± 0.5	-7.5 ± 0.6	-27 ± 2
	9.4	$1.02 \pm 0.06 \times 10^4$	21.7 ± 0.1	15.2 ± 0.3	-6.5 ± 0.3	-23 ± 1
	25.0	$4.37 \pm 0.43 \times 10^3$	20.8 ± 0.3	14.6 ± 0.5	-6.2 ± 0.6	-21 ± 2
	25.2	$6.89 \pm 0.57 \times 10^3$	21.9 ± 0.2	17.8 ± 0.4	-4.1 ± 0.5	-14 ± 2
Average value:				15.6 ± 1.0		-21 ± 5
1HGlu	5.8	531 ± 19	14.6 ± 0.1	17.7 ± 0.4	-3.1 ± 0.4	11 ± 1
	6.6	525 ± 16	14.6 ± 0.1	17.1 ± 0.3	-2.5 ± 0.3	9 ± 1
	23.9	344 ± 7	14.4 ± 0.1	17.4 ± 0.2	-3.0 ± 0.2	10 ± 1
	24.7	347 ± 8	14.5 ± 0.1	18.4 ± 0.2	-2.9 ± 0.2	10 ± 1
Average value:				17.7 ± 0.5		10 ± 1
1FGlu	8.3	175 ± 7	12.1 ± 0.1	29.5 ± 0.9	17.4 ± 0.9	62 ± 3
	9.7	172 ± 11	12.0 ± 0.2	33.8 ± 1.4	21.8 ± 1.4	77 ± 5
	9.9	167 ± 10	12.0 ± 0.2	34.5 ± 1.4	22.5 ± 1.4	80 ± 5
	23.7	148 ± 7	12.3 ± 0.1	31.5 ± 0.8	19.2 ± 0.8	65 ± 3
Average value:	24.9	145 ± 9	12.3 ± 0.1	28.2 ± 1.2	15.9 ± 1.2	$53 \pm 4^*$
				31.5 ± 2.7		71 ± 9
2HGlu	7.7	452 ± 18	14.3 ± 0.1	20.2 ± 0.4	5.9 ± 0.4	21 ± 1
	8.4	467 ± 13	14.4 ± 0.1	21.5 ± 0.3	7.1 ± 0.3	25 ± 1
	23.8	434 ± 10	15.0 ± 0.1	20.7 ± 0.2	5.7 ± 0.2	19 ± 1
	24.8	470 ± 14	15.2 ± 0.1	20.7 ± 0.3	5.5 ± 0.3	19 ± 1
Average value:				20.8 ± 0.5		21 ± 3
2FGlu	8.8	523 ± 8	14.7 ± 0.1	27.1 ± 0.2	12.4 ± 0.2	44 ± 1
	9.7	526 ± 8	14.7 ± 0.1	27.0 ± 0.2	12.3 ± 0.2	44 ± 1
	23.3	379 ± 12	14.6 ± 0.1	26.8 ± 0.5	12.2 ± 0.5	41 ± 2
	24.4	448 ± 10	15.1 ± 0.1	24.6 ± 0.3	9.5 ± 0.3	32 ± 1
Average value:	24.8	455 ± 13	15.2 ± 0.1	$22.6 \pm 0.4^*$	7.4 ± 0.4	$25 \pm 1^*$
				26.4 ± 1.2		40 ± 5
3FGlu	9.0	253 ± 11	13.0 ± 0.1	16.0 ± 0.4	3.0 ± 0.4	11 ± 1
	9.3	283 ± 7	13.3 ± 0.1	15.9 ± 0.2	2.6 ± 0.2	9 ± 1
	10.2	245 ± 8	13.0 ± 0.1	18.5 ± 0.4	5.5 ± 0.4	19 ± 1
	23.5	215 ± 6	13.2 ± 0.1	18.2 ± 0.3	5.0 ± 0.3	17 ± 1
Average value:	24.1	194 ± 8	13.0 ± 0.1	16.4 ± 4.7	3.4 ± 4.7	0 ± 6
				17.0 ± 1.3		14 ± 5

* This value was excluded when determining the average value.

calorimetric enthalpies was observed for 1HGlu; for 3FGlu binding to conA; for α PhOGlu, 1FGlu, 2FGlu, 3FGlu and 3MeOGlu binding to pea lectin; and for 2HGlu, 2FGlu and 3MeOGlu binding to lentil lectin. The van't Hoff enthalpies for the other binding reactions were all lower than the calorimetric enthalpies.

Because ΔG_b^0 is a function of the difference between ΔH_b^0 and $T\Delta S_b^0$, differences in ΔH_b^0 and ΔS_b^0 between the derivatives and Glu are presented in Table 4 for comparison. Differences in two calculated uncertainties are considered to be zero in Table 4. The binding enthalpies of Glu are -16.6 ± 0.5 kJ·mol⁻¹ to conA, -14.5 ± 1.2 kJ·mol⁻¹ to pea lectin and -9.7 ± 0.9 kJ·mol⁻¹ to lentil lectin, whereas the binding entropies of Glu are zero except for binding to lentil lectin, which is -13 ± 4 J·mol⁻¹·K⁻¹ [5].

DISCUSSION

To correlate differences in the thermodynamic binding parameters with changes in the interactions between the carbohydrate derivatives and the amino acid residues at the binding site, any conformational differences between the derivatives and the effects of desolvation of both the derivative and the lectin binding site have to be taken into consideration. Replacement of an OH group with a substituent such as H or F, or a bulky one such as MeO- on a six-membered ring, has little effect on its conformation in aqueous solution [12]. For example, NMR

measurements on solutions of 2FGlu by Adamson et al. [13] and on 3FGlu by Foster et al. [14] indicate that the F is in the same position, i.e. equatorial to the ring, as was the OH group. For the C-1-substituted derivatives, the anomeric effect, i.e. the tendency of the group to assume an axial position, is enhanced with replacement of the OH on C-1 with F [15]. Thus this substitution would favour the α -configuration in the equilibrium between the α and β conformers in solution. Overall there is strong evidence that the conformations of the Glu derivatives are the same in aqueous solutions [9].

Differences in the thermodynamic binding parameters could result from differences in their desolvation thermodynamic parameters. There is evidence for the formation of hydration complexes between water and Glu and Man [16] and between water and α MeOGlu [17] in aqueous solutions, because the equatorial OH oxygens on the same side of the pyranose ring are 0.482 nm apart in the chair conformation [16], which corresponds to high concentrations of water molecules 0.49 nm apart observed in X-ray diffraction studies [18]. Shio [17] estimated that the enthalpy of formation for the hydration complexes is about -54 kJ·mol⁻¹. If desolvation contributes substantially to the binding thermodynamics, there should be large differences in the binding enthalpies between Glu, Man and α MeOGlu and the deoxy derivatives of Glu, which are less likely to form hydrates with water because one of their equatorial hydroxy groups is replaced with H. The small differences in the binding enthalpies

Table 2 Thermodynamic binding parameters of glucopyranoside derivatives to pea lectin

	Temp. (°C)	K_b (M ⁻¹)	$-\Delta G^0$ (kJ·mol ⁻¹)	$-\Delta H_b^0$ (kJ·mol ⁻¹)	$-T\Delta S_b^0$ (kJ·mol ⁻¹)	$-\Delta S_b^0$ (J·mol ⁻¹ ·K ⁻¹)
α PhOGlu	8.6	955 ± 53	16.1 ± 0.1	12.5 ± 1.0	-3.6 ± 1.0	-13 ± 4
	7.0	1.19 ± 0.12 × 10 ³	16.5 ± 0.2	9.8 ± 0.4	-6.7 ± 0.2	-24 ± 1
	24.0	970 ± 44	17.0 ± 0.1	10.1 ± 0.3	-6.9 ± 0.3	-23 ± 1
	24.4	798 ± 31	16.5 ± 0.1	12.5 ± 0.3	-4.0 ± 0.3	-13 ± 1
Average value:				11.2 ± 1.5		-18 ± 6
1FGlu	3.9	182 ± 14	12.0 ± 0.2	17.9 ± 0.9	5.9 ± 0.9	21 ± 3
	4.6	182 ± 4	12.0 ± 0.1	18.3 ± 0.3	6.3 ± 0.3	23 ± 1
	23.7	110 ± 6	11.6 ± 0.1	18.1 ± 0.6	6.5 ± 0.6	22 ± 2
	24.0	136 ± 9	12.1 ± 0.2	16.3 ± 0.7	4.2 ± 0.7	14 ± 2
Average value:				17.7 ± 0.9		20 ± 4
1FGlu	7.6	194 ± 4	12.3 ± 0.1	18.1 ± 2.0	5.8 ± 2.0	21 ± 7
	7.7	187 ± 13	12.2 ± 0.2	17.8 ± 0.8	5.6 ± 0.8	20 ± 3
	24.0	140 ± 8	12.2 ± 0.1	18.2 ± 0.7	6.0 ± 0.7	20 ± 2
	25.3	131 ± 3	12.1 ± 0.1	18.4 ± 0.2	6.3 ± 0.2	21 ± 1
Average value:				18.1 ± 0.3		21 ± 1
2HGlu	9.1	215 ± 16	12.6 ± 0.2	20.7 ± 0.9	8.1 ± 0.9	29 ± 3
	10.0	184 ± 9	12.3 ± 0.1	19.7 ± 0.6	7.4 ± 0.6	26 ± 2
	24.4	171 ± 3	12.7 ± 0.1	23.3 ± 0.3	10.6 ± 0.3	36 ± 1
	24.5	152 ± 6	12.4 ± 0.1	23.6 ± 0.6	11.2 ± 0.4	38 ± 1
2FGlu	24.9	190 ± 9	13.1 ± 0.1	21.5 ± 0.7	9.4 ± 0.7	32 ± 2
				21.8 ± 1.7		32 ± 5
	7.5	625 ± 27	15.0 ± 0.1	15.4 ± 1.2	0 ± 1	0 ± 1
	11.5	568 ± 10	15.0 ± 0.1	15.9 ± 0.1	0 ± 0.1	0 ± 0.1
2FGlu	24.6	388 ± 17	14.8 ± 0.1	16.4 ± 0.4	1.6 ± 0.4	5 ± 1
	24.9	308 ± 9	14.2 ± 0.1	20.8 ± 0.4*	6.6 ± 0.4	22 ± 1
				15.9 ± 0.5		0 ± 3
				12.4 ± 2.4	0 ± 2	0 ± 2
3FGlu	8.4	140 ± 7	11.6 ± 0.1	15.9 ± 2.4	4.4 ± 2.4	16 ± 8
	9.9	133 ± 3	11.5 ± 0.1	15.9 ± 2.4	4.4 ± 2.4	16 ± 8
	24.2	123 ± 12	11.9 ± 0.2	11.9 ± 0.9	0 ± 1	0 ± 1
	24.5	117 ± 15	11.8 ± 0.3	14.9 ± 1.5	3.1 ± 1.5	10 ± 5
Average value:				13.8 ± 1.9		0 ± 10
3MeOGlu	6.5	3.75 ± 0.48 × 10 ³	19.1 ± 0.3	14.1 ± 0.7	-5.0 ± 0.7	-18 ± 3
	7.8	4.50 ± 0.23 × 10 ³	19.6 ± 0.1	13.5 ± 0.3	-6.1 ± 0.3	-22 ± 1
	23.7	3.12 ± 0.12 × 10 ³	19.9 ± 0.1	14.1 ± 0.2	-5.8 ± 0.2	-20 ± 1
	24.0	2.47 ± 0.01 × 10 ³	19.3 ± 0.1	13.8 ± 0.3	-5.5 ± 0.3	-19 ± 1
Average value:				13.9 ± 0.5		-19 ± 2

* This value was excluded when determining the average values.

between Glu and the deoxy derivatives in Table 4 show that this is not so. From differences in the binding enthalpies of α MeOMan to conA in H₂O and ²H₂O, Chervenak and Toone [19] showed that solvent reorganization may contribute about 7% to the binding enthalpy. X-ray crystallography studies of the α MeOMan–conA complex [10] show that the methyl group rotates freely in the solvent and there are interactions between a water molecule and C-2 hydroxy group of the mannopyranoside ring. This implies that substituents at C-1 and C-2 remain exposed to the solvent on binding to conA, and thus the possibility of desolvation contributing to the binding parameters is minimal. The X-ray crystal structure of pea lectin complexed with a trimannoside shows that the binding site is similar to that of conA and that neither the C-1 nor the C-2 hydroxy groups are hydrogen bonded to the protein [20], implying that, again, substituents at these positions remain exposed to the solvent upon binding. The lectin desolvation thermodynamic parameter should be the same for all the Glu derivatives binding to a particular lectin since it relates to bond breakage between water molecules and the amino acid residues only at the binding site. Accordingly, in the following discussion, desolvation contributions to the binding process will not be considered.

The average ΔH_b^0 and ΔS_b^0 values in Tables 1–3 exhibit some general characteristics. Comparison of the binding enthalpies at

approx. 10 °C with the binding enthalpies at room temperature show that the binding enthalpies are invariant with regard to temperature, as observed previously [5,7,8]. Earlier indirect determinations of the binding enthalpies for several lectin–sugar interactions have also assumed that $\Delta C_p = 0$, and some of these have now been validated by calorimetric studies [7,21]. The enthalpically driven nature of the binding reaction between the Glu derivatives and the lectins is maintained although there are negative and positive ΔS_b^0 contributions to ΔG_b^0 . Enthalpy and entropy compensation is also observed, as shown in Figure 2, so that changes in the enthalpy are compensated for by changes in the entropy, as discussed previously [5].

Observation of enthalpy–entropy compensation is not surprising as thermodynamic studies on several lectins, from both legumes and other sources, strongly emphasize the importance of water as a mediator in protein–carbohydrate recognition [21–26]. This fact is also succinctly brought out by the high-resolution structural analysis of *Lathyrus ochrus* lectin I complexed with its complementary oligosaccharide ligand [27]. Other factors, although less likely, could involve subtle shifts in protein conformational states and cannot be ignored as a source of the observed enthalpy–entropy compensation [28].

Agreement between the van't Hoff binding enthalpies and the calorimetric enthalpies is observed for only 60% of the binding

Table 3 Thermodynamic binding parameters of glucopyranoside derivatives to lentil lectin

	Temp. (°C)	K_b (M ⁻¹)	$-\Delta G_b^0$ (kJ·mol ⁻¹)	$-\Delta H_b^0$ (kJ·mol ⁻¹)	$-T\Delta S_b^0$ (kJ·mol ⁻¹)	$-\Delta S_b^0$ (J·mol ⁻¹ ·K ⁻¹)
α PhOGlu:	7.7	350 ± 19	13.7 ± 0.1	11.0 ± 0.4	-2.7 ± 0.4	-10 ± 1
	7.8	281 ± 22	13.2 ± 0.2	11.3 ± 0.6	-1.9 ± 0.6	-7 ± 2
	24.0	391 ± 10	14.7 ± 0.1	12.4 ± 0.2	-2.3 ± 0.2	-8 ± 1
	24.6	285 ± 10	14.0 ± 0.1	11.6 ± 0.6	-2.4 ± 0.6	-8 ± 2
Average value:				11.6 ± 0.6		-8 ± 1
1HGlu	6.2	81.3 ± 6.0	10.2 ± 0.2	14.9 ± 0.7	4.7 ± 0.7	17 ± 3
	7.0	75.0 ± 6.0	9.8 ± 0.2	14.1 ± 0.8	4.3 ± 0.8	15 ± 3
	23.7	80.6 ± 3.0	10.8 ± 0.1	15.5 ± 0.4	4.7 ± 0.4	16 ± 1
	24.8	56.0 ± 3.0	10.0 ± 0.1	14.9 ± 0.6	4.9 ± 0.6	16 ± 2
Average value:				14.9 ± 0.6		16 ± 1
1FGlu	8.1	86.3 ± 4.9	10.4 ± 0.1	24.7 ± 1.0	14.3 ± 1.0	51 ± 4
	9.4	76.0 ± 3.5	10.2 ± 0.1	23.4 ± 1.0	13.2 ± 1.0	47 ± 4
	23.6	74.9 ± 7.6	10.6 ± 0.2	28.9 ± 2.3	18.3 ± 2.3	62 ± 8
	24.3	72.5 ± 4.1	10.6 ± 0.1	25.2 ± 1.0	14.6 ± 1.0	49 ± 3
Average value:				25.6 ± 2.4		52 ± 7
2HGlu	8.4	102 ± 3	10.8 ± 0.1	17.9 ± 0.4	7.1 ± 0.4	25 ± 1
	9.7	110 ± 4	11.1 ± 0.1	15.6 ± 0.3	4.5 ± 0.3	16 ± 1
	24.7	80 ± 4	10.9 ± 0.1	16.4 ± 0.4	5.5 ± 0.4	18 ± 1
	25.0	100 ± 3	11.4 ± 0.1	17.9 ± 0.4	6.5 ± 0.4	22 ± 1
Average value:				17.0 ± 1.2		20 ± 4
2FGlu	8.5	238 ± 5	12.8 ± 0.1	15.4 ± 0.2	2.6 ± 0.2	9 ± 1
	8.8	248 ± 9	12.9 ± 0.1	16.1 ± 0.4	2.2 ± 0.4	8 ± 1
	24.1	174 ± 4	12.7 ± 0.1	17.9 ± 2.3	5.2 ± 2.3	17 ± 8
	24.1	199 ± 5	13.1 ± 0.1	17.9 ± 0.2	4.8 ± 0.2	16 ± 1
Average value:				16.8 ± 1.3		13 ± 5
3FGlu	8.5	52.6 ± 4.1	9.3 ± 0.2	14.8 ± 0.8	5.5 ± 0.8	20 ± 3
	9.1	49.4 ± 2.8	9.2 ± 0.1	13.7 ± 0.5	4.5 ± 0.5	16 ± 2
	24.2	48.5 ± 1.8	9.6 ± 0.1	14.9 ± 0.4	5.3 ± 0.4	18 ± 1
	24.9	47.7 ± 3.6	9.6 ± 0.2	15.9 ± 0.8	6.3 ± 0.8	21 ± 3
Average value:				14.8 ± 0.9		19 ± 2
3MeOGlu	7.6	3.09 ± 0.09 × 10 ³	18.8 ± 0.1	12.0 ± 0.1	-6.8 ± 0.1	-24
	9.0	2.48 ± 0.81 × 10 ³	18.3 ± 0.8	10.8 ± 0.2	-7.5 ± 0.8	-26 ± 3
	9.3	1.86 ± 0.19 × 10 ³	17.7 ± 0.2	11.7 ± 0.5	-6.0 ± 0.5	-21 ± 2
	23.6	1.66 ± 0.05 × 10 ³	18.3 ± 0.1	15.1 ± 0.2	-3.2 ± 0.2	-11 ± 1
Average value:				15.1 ± 0.2	-3.6 ± 0.2	-12 ± 1
				13.5 ± 1.9		-17 ± 5

Table 4 Summary of differences in the thermodynamic parameters between D-glucopyranoside derivatives at 24–25 °C

Abbreviation used: n.r., not recorded.

Derivative	Concanavalin A		Pea lectin		Lentil lectin	
	$-\Delta\Delta H_b^0$ (kJ·mol ⁻¹)	$-\Delta\Delta S_b^0$ (J·K ⁻¹ ·mol ⁻¹)	$-\Delta\Delta H_b^0$ (kJ·mol ⁻¹)	$-\Delta\Delta S_b^0$ (J·K ⁻¹ ·mol ⁻¹)	$-\Delta\Delta H_b^0$ (kJ·mol ⁻¹)	$-\Delta\Delta S_b^0$ (J·K ⁻¹ ·mol ⁻¹)
α MeOGlu	2.2 ± 0.9	0	0	-11 ± 3	0	-11 ± 4
α PhOGlu	0	-25 ± 3	0	-18 ± 6	0	0
3MeOGlu	n.r.	n.r.	0	-19 ± 2	0	0
1HGlu	0	10 ± 1	3.2 ± 1.5	20 ± 4	5.2 ± 0.9	29 ± 4
1FGlu	14.9 ± 2.7	71 ± 9	3.6 ± 1.2	21 ± 1	15.9 ± 2.6	65 ± 8
2HGlu	4.2 ± 0.7	21 ± 3	7.3 ± 2.1	32 ± 5	7.3 ± 1.5	33 ± 6
2FGlu	9.8 ± 1.3	40 ± 6	0	0	7.1 ± 1.6	26 ± 6
Man	4.5 ± 0.9	14 ± 4	10.7 ± 1.3	29 ± 8	3.8 ± 0.9	0
3HGlu	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
3FGlu	0	14 ± 5	0	0	5.1 ± 1.3	32 ± 5
4HGlu	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
4FGlu	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
6HGlu	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
6FGlu	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.

Figure 2 Plot of $T\Delta S^\circ_b$ as a function of ΔH°_b for the derivatives binding to conA, pea lectin and lentil lectin

The line follows the equation $T\Delta S^\circ_b = 15.7 \pm 2.2 + 1.08 \pm 0.12\Delta H^\circ_b$, where T is in K and $T\Delta S^\circ_b$ and ΔH°_b are in $\text{kJ} \cdot \text{mol}^{-1}$.

reactions. This lack of agreement has been observed by Naghibi et al. [29] for a variety of other binding interactions such as the binding of cyclohexanol to α - and β -cyclodextrins. The concentrations of the reactants for these binding reactions were low enough for deviations of these solutions from ideal behaviour to be negligible. This lack of agreement was therefore tentatively attributed to contributions to ΔH°_b from changes with temperature in other equilibria between the solutes and/or water, buffer, etc., occurring in solution and not accounted for by the simple binding reaction.

Differences in the enthalpies and entropies between the derivatives and Glu presented in Table 4 show the importance of single group substitutions on the binding thermodynamics of carbohydrates to lectins. Differences in ΔH°_b can be used to classify the derivatives into three categories: non-binding derivatives, derivatives with $\Delta\Delta H^\circ_b = 0$, and derivatives with $\Delta\Delta H^\circ_b < 0$. Replacement of the OH group with F and H on C-4 and on C-6 apparently eliminates binding of the Glu derivatives to the lectins, as was observed by van Wauwe et al. [3] for 4FGlu and 6FGlu binding to pea lectin. Site-specific mutagenesis studies [30] have indicated that stronger hydrogen bonds are formed between charged and neutral atoms than between two neutral atoms. An examination of the structures of conA- α MeOMan and pea lectin-trimannopyranoside indicates that the equatorially oriented hydroxy groups at C-4 and C-6 of Man are involved in donating hydrogen bonds to atoms emanating from charged residues in both conA and pea lectin. Because the recently solved structure of lentil lectin shows identical residues at an analogous position to that in pea lectin, it is reasonable to assume that contacts similar to those observed in the pea lectin-carbohydrate complex also operate in a lentil lectin-monosaccharide complex. According to these expectations, the greatest loss in binding enthalpy should have occurred with 4HGluc and 6HGluc, as is indeed observed experimentally.

In contrast with 3FGlu, 3HGluc does not bind to the lectins, which substantiates the observation that the C-3 OH can accept a hydrogen bond from the NH group of Arg-228 of conA [10] as well as from Gly-99 of pea lectin [20]. The lack of binding between 3MeOgluc and conA may result from steric hindrance of the methoxy group on C-3 in the conA binding pocket. Overlapping of the liganded binding site of pea lectin with that of conA reveals that Arg-228 of conA occupies the corresponding

position of Gly-99 of pea lectin [20]. The C-3 OCH_3 group would be sterically hindered by the side chain of Arg-228 of conA and not the smaller side chain of Gly-99 of pea lectin [20].

It is observed that $\Delta\Delta H^\circ_b = 0$ for 1Hgluc and 3FGlu binding to conA, for α MeOgluc, 3FGlu and 3MeOgluc binding to pea, for α MeOgluc and 3MeOgluc binding to lentil lectin and for α PhOgluc binding to all three lectins. Despite the lack of a change in ΔH°_b , there is an increase in the binding affinity ($-\Delta G^\circ_b$) for α PhOgluc binding to conA and pea lectin, for 3MeOgluc binding to pea lectin and for α MeOgluc binding to pea and lentil lectin because of an increase in the binding entropy relative to α Glu. Because methyl and phenyl groups can form hydrophobic bonds with the amino acid residues, the entropy increases could be indicative of hydrophobic interactions between these groups and pea lectin as well as between α MeOgluc and lentil lectin. For binding of α PhOgluc to conA and to pea lectin, a hydrophobic interaction could result from an aromatic stacking interaction with the neighbouring aromatic ring of Tyr-12 in conA and Phe-123 in pea lectin at the binding site. For binding of 3MeOgluc to pea lectin, X-ray crystallographic studies [20] have led to the speculation that the proximity of the C-3 OCH_3 group to Tyr-100 and Trp-128 in pea lectin could lead to a favourable hydrophobic interaction. This is now shown by the additional entropy increase on 3MeOgluc binding to pea lectin relative to α MeOgluc binding to pea lectin.

Increases in the binding enthalpy occur for α MeOgluc binding to conA, 1Hgluc binding to pea and lentil lectin, for 1FGlu, 2Hgluc and Man binding to all three lectins, and for 2FGlu binding to conA and lentil lectin. In binding to conA, replacement of the OH group on C-1 and C-2 with F yields an enthalpy change almost twice as great as for α Glu binding. In the crystal complex between conA and α MeOMan [10], hydrogen bond interactions between either the methoxy group on C-1 or the hydroxy group on C-2 and the amino acid residues at the binding site were not observed, although the OH group on C-2 forms a link through a water bridge to an adjacent conA molecule. The large enthalpy change associated with replacement of OH on C-1 and C-2 with F or H may be associated with weaker interactions with the adjacent solvent water molecules of the complex, further stabilizing the complex. This is substantiated by the large decrease in the entropy difference with Glu, $\Delta\Delta S^\circ_b < 0$, except for Man binding to lentil lectin. The closeness of the $\Delta\Delta H^\circ_b$ and $\Delta\Delta S^\circ_b$ values for 2Hgluc to those for Man binding to conA and pea lectin implies that removal of the OH from the equatorial position in Glu by replacement with H or movement to an axial position enhances the binding interaction almost to the same extent for binding to conA and pea lectin. The stronger binding interaction of conA to α MeOgluc is perhaps due to an additional non-polar interaction between Leu-99 and the methyl group of α MeOgluc. In pea and lentil lectin, an alanine residue (Ala-212) at the position corresponding to Leu-99 would reduce this non-polar interaction and thus $\Delta\Delta H^\circ_b = 0$ for α MeOgluc binding to pea and lentil lectin.

Changes in the thermodynamic parameters caused by replacements of the OH groups on the pyranoside ring of Gluc can be explained qualitatively in terms of the α MeOMan-conA crystal complex determined by Derewenda et al. [10] and the pea lectin-trimannoside complex determined by Rini et al. [20]. This implies that there is very little variation in the overall structure of the conA complex after replacement of the OH groups. Determination of the contributions of the binding enthalpy and entropy changes to the binding affinities for the derivatives is important in developing a qualitative model for the binding interactions. Effects of the various substitutions can be more easily assessed quantitatively by performing molecular docking

computations such as those of Imberty et al. [31] on binding to conA with the substituted Glu derivatives. Such computations are now in progress.

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