Thermodynamics of Carbohydrate Binding to Galectin-1 from Chinese Hamster Ovary Cells and Two Mutants. A Comparison with Four Galactose-Specific Plant Lectins[†]

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ABSTRACT: The thermodynamics of carbohydrate binding to the 14 kDa dimeric β-galactoside-binding lectin galectin-1 (Gal-1) from Chinese hamster ovary cells and four galactose-specific plant lectins were investigated by isothermal titration microcalorimetry. Recombinant Gal-1 from *Escherichia coli*, a Cys→Ser mutant with enhanced stability (C2S-Gal-1), and a monomeric mutant of the lectin (*N*-Gal-1) were studied along with the soybean agglutinin and the lectins from *Erythrina indica*, *Erythrina crystagalli*, and *Erythrina corollodendrum*. Although the pattern of association constants of the *Erythrina* lectins was similar for mono- and disaccharides, variations exist in their enthalpy of binding ($-\Delta H$) values for individual carbohydrates. While the *Erythrina* lectins show greater affinities and $-\Delta H$ values for lactose and *N*-acetyllactosamine, the soybean agglutinin possesses similar affinities for methyl β-galactopyranoside, lactose, and *N*-acetyllactosamine and a greater $-\Delta H$ value for the monosaccharide. Gal-1 and the plant lectins possess essentially the same affinities for *N*-acetyllactosamine; however, the animal lectin shows a lower $-\Delta H$ value and more favorable binding entropy for the disaccharide. While Gal-1, C2S-Gal-1, and *N*-Gal-1 all possess essentially the same affinities for *N*-acetyllactosamine, the two mutants possess much lower $-\Delta H$ values, even though the mutation site(s) are far removed from the carbohydrate binding site. These results indicate that there are different energetic mechanisms of carbohydrate binding between galectin-1, its two mutants, and the Gal-specific plant lectins.

Lectins are carbohydrate binding proteins of non-immune origin which are widely distributed in nature [cf. Lis and Sharon (1991)]. Lectin binding to the carbohydrate moieties of glycoproteins and glycolipids has been implicated in a variety of biological processes, including cellular adhesion (Moore et al., 1991), cellular recognition (McEver et al., 1995), protein folding (Helenius, 1994) and signal transduction [cf. Brandley and Schnaar (1986), Lasky (1992), and Perillo et al. (1995)]. Lectins have also been used as tools for studying glycosylation mutants (Stanley, 1993), separation and purification of polysaccharides, glycopeptides, and glycoproteins (Cummings, 1994), and probing normal and tumor cell membrane structures and dynamics (Roth et al., 1994). Lectin−carbohydrate interactions have also been associated with a variety of pathogenic processes (Lasky, 1992; Raz et al., 1990; Sauter et al., 1992; Sharon & Lis, 1989). Thus, there is considerable interest in understanding the molecular basis of the specificity of carbohydrate−lectin interactions in biological systems.

In order to understand the carbohydrate binding specificities of lectins, the thermodynamics of binding must be determined. Lectin affinity differences can then be understood in terms of the relative contributions of the enthalpy (ΔH) and the entropy ($T\Delta S$) of binding for each carbohydrate. The relative magnitude of ΔH is usually associated with ligand−protein interactions such as hydrogen bonding, ionic bonding and positive van der Waals interactions (Hinz, 1983). The magnitude of the $T\Delta S$ term is associated with solvent reorganization and other entropic contributions to binding (Hinz, 1983). The "size" of the combining site of a lectin can therefore be determined, to a good approximation, from the *relative* magnitude of ΔH for a series of carbohydrates [cf. Bains et al. (1992) and Mandal et al. (1994)]. Furthermore, changes in the heat capacity of binding (ΔC_p) provides another thermodynamic parameter to characterize each lectin−carbohydrate complex (Sturtevant, 1977).

In the present study, the thermodynamics of carbohydrate binding to a dimeric β-galactoside-binding lectin (Gal-1)

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[1] Abbreviations: Gal-1, the dimeric β-galactoside-binding 14 kDa lectin from Chinese hamster ovary cells; C2S-Gal-1, a serine for cysteine mutant at position 2 of Gal-1; *N*-Gal-1, a 4-substituted mutant of galectin-1 involving a serine for cysteine mutation at position 2, glutamine for leucine at position 4, aspartic acid for valine at position 5, and serine for alanine at position 6; SBA, soybean agglutinin *(Glycine max)*; EIL, lectin from *Erythrina indica*; ECL, lectin from *Erythrina cristagalli*; ECorL, lectin from *Erythrina corallodendron*; LacNAc, Galβ(1,4)GlcNAc; MeβGal; methyl β-galactopyranoside; GalNAc, *N*-acetylgalactamine. All sugars are in the D configuration.

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from Chinese hamster ovary cells (CHO), a C2S mutant with enhanced stability, and a monomeric mutant of the lectin (*N*-Gal-1) have been determined by isothermal titration microcalorimetry. The results have been compared with the thermodynamics of carbohydrate binding to four Gal-specific plant lectins: soybean agglutinin from *Glycine max* (SBA), *Erythrina indica* lectin (EIL), *Erythrina crystagalli* lectin (ECL), and *Erythrina corollodendrum* lectin (ECorL). Previous studies using hemagglutination−inhibition assays have shown that EIL, ECL, and ECorL show higher affinities for LacNAc than other mono- and disaccharides (Bhattacharyya et al., 1988, 1989) and that SBA possesses higher affinity for GalNAc than other mono- and disaccharides (Hammarstrom et al., 1977; Pereira et al., 1974). Binding studies of the 14 kDa Gal-specific family of lectins have shown a specificity for LacNAc [cf. Abbott et al. (1988), Ahmed et al. (1990), Gupta et al. (1993), and Lee et al. 1990)].

X-ray crystal structures of several of the lectins have been reported. The crystal structure at 2.0 Å of ECorL complexed with lactose (Shaanan et al., 1991) and the X-ray structure at 2.6 Å of SBA cross-linked with a biantennary pentasaccharide have recently been reported (Dessen et al., 1995). The crystal structure at 1.9 Å of Gal-1 from bovine spleen complexed with LacNAc (Liao et al., 1994), three crystal structures at 2.15−2.45 Å of Gal-1 from bovine muscle complexed with a biantennary complex carbohydrate (Bourne et al., 1994), and the crystal structure at 2.9 Å of human galectin-2 complexed with lactose (Lobsanov et al., 1993) have been reported.

The present thermodynamic binding data are discussed in terms of the carbohydrate binding specificities of the lectins as well as a comparison of the energetics of binding of the plant and animal lectins to carbohydrates.

MATERIALS AND METHODS

EIL, ECL, and ECorL were prepared according to the procedures described earlier (Bhattacharyya et al., 1981; Iglesias et al., 1982; Lis et al., 1985). SBA was purified as previously described (Bhattacharyya et al., 1988). Recombinant Gal-1 from CHO cells and C2S-Gal-1 (Cho & Cummings, 1995a) as well as a monomeric 4-substituted mutant of Gal-1 (*N*-Gal-1) possessing a serine for cysteine mutation at position 2, glutamine for leucine at position 4, aspartic acid for valine at position 5, and serine for alanine at position 6 (Cho and Cummings, in press) have been recently prepared. MeβGal, lactose, LacNAc, and GalNAc were purchased from Sigma Chemical Company (St. Louis, MO). MeβLacNAc was a gift from Dr. S. Sabesan, Dupont Company (Wilmington, DE). The concentrations of the carbohydrates were measured using a modified phenol−sulfuric acid method (Saha & Brewer, 1994) with Gal as the standard. The concentration of the lectins were determined by Lowry estimation using BSA as the standard.

Titration Calorimetry. Isothermal titration microcalorimetry (Figure 1) was performed using an OMEGA Microcalorimeter from Microcal, Inc. (Northampton, MA). In individual titrations, injections of 3 or 4 μL of carbohydrate were added from the computer-controlled 100 μL microsyringe at an interval of 4 min into the lectin solution (cell volume = 1.3424 mL) dissolved in the same buffer as the saccharide, while stirring at 350 rpm. Control experiments performed by making identical injections of saccharide into a cell containing buffer with no protein showed insignificant heats of dilution. The experimental data were fitted to a theoretical titration curve using software supplied by Microcal, with ΔH (the enthalpy change in kcal mol^{-1}), K_a (the association constant in M^{-1}), and n (the number of binding sites per monomer) as adjustable parameters. The quantity $c = K_a M_t(0)$, where $M_t(0)$ is the initial macromolecule concentration, is of importance in titration microcalorimetry (Wiseman et al., 1989). All experiments were performed with c values $1 < c < 200$ in the present study. The instrument was calibrated by using the calibration kit containing ribonuclease A (RNase A) and cytidine 2′-monophosphate (2′-CMP) supplied by the manufacturer.

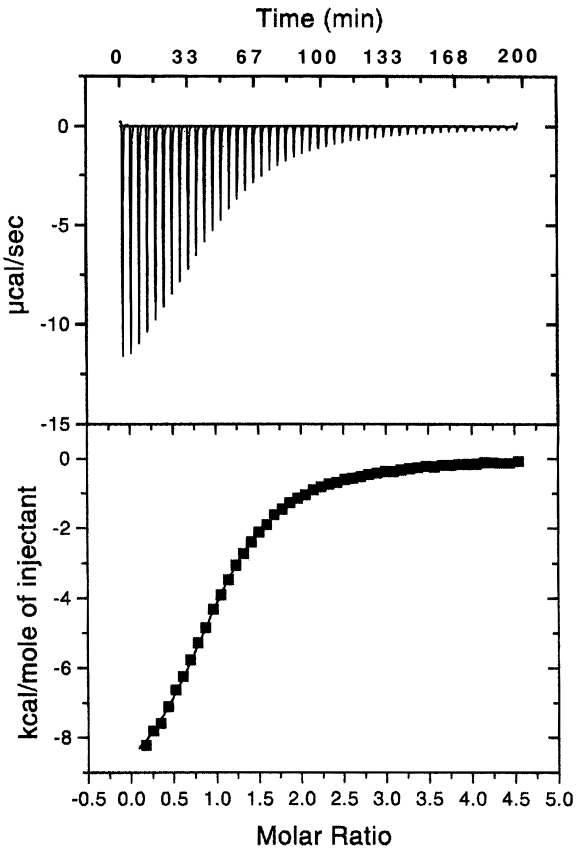

FIGURE 1: Titration microcalorimetric data for EIL (0.36 mM) with MeβLacNAc (10.5 mM) at 25 °C. (A) Raw data obtained from 50 automatic injections, 4 μL each, of MeβLacNAc. and (B) integrated curve showing experimental points (■) and the best fit (---). The buffer was 0.1 M Hepes buffer with 0.15 M NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$.

The thermodynamic parameters were calculated form basic equations of thermodynamics:

$$\Delta G = \Delta H - T\Delta S = -RT \ln K_a$$

where ΔG, ΔH and ΔS are the changes in free energy, enthalpy, and entropy of binding, respectively. T is the absolute temperature, and $R = 1.98$ cal/mol K.

The change in heat capacity of binding (ΔC_p), which is assumed to be independent of temperature, was determined using the following expression:

$$\Delta C_p = \Delta H_{T_2} - \Delta H_{T_1}/T_2 - T_1$$

Scatchard Analysis. Scatchard plots (Figure 2) were obtained from the titration microcalorimetry data as previously described (Mandal et al., 1994).

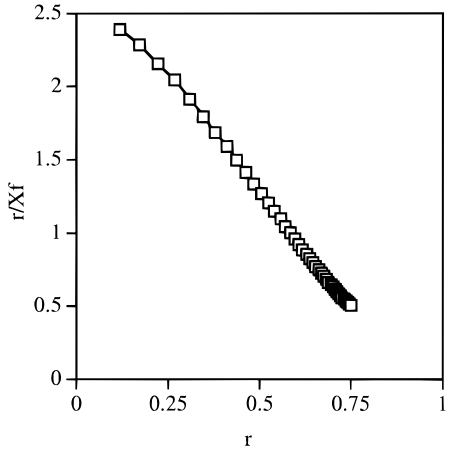

FIGURE 2: Scatchard plot for the binding of C2S-Gal-1 mutant (0.28 mM) with LacNAc (26 mM) at pH 7.2 and 27 °C. The values of r and r/X_f were calculated using the equations described in the Materials and Methods. r/X_f is expressed in the terms of M^{-1}.

RESULTS AND DISCUSSION

The isothermal titration microcolorimetry data for binding of mono- and disaccharides to four Gal-specific plant lectins and recombinant Gal-1 from CHO cells and two mutants are shown in Table 1. The results are discussed in terms of the carbohydrate binding specificities of the lectins and differences in the mechanisms of carbohydrate binding to plant and animal lectins.

Properties of the Lectins. SBA is a tetrameric Gal/GalNAc-specific lectin of molecular mass 120 kDa with one carbohydrate binding site per monomer (De Boeck et al., 1984a). SBA, like most legume lectins, requires bound Ca^{2+} and Man^{2+} for its carbohydrate binding activity (Goldstein & Poretz, 1986). SBA exhibits greatest affinity for GalNAc among monosaccharides (Lis et al., 1970; Pereira et al., 1974), and hemagglutination inhibition assays indicate that MeβGal, lactose, and LacNAc bind to the lectin with nearly the same affinities (Bhattacharyya et al., 1988).

EIL, ECL, and ECorL are dimeric lectins that possess close physicochemical and carbohydrate binding properties (Bhattacharyya et al., 1981, 1989). The molecular masses of ECL and ECorL are 56 kDa and 68 kDa for EIL. All three are Gal-specific lectins with one carbohydrate binding site per monomer. Like all legume lectins, they require Mn^{2+} and Ca^{2+} for their carbohydrate binding activities (Bhattacharyya et al., 1981; Iglesias et al., 1982; Lis et al., 1985). LacNAc among simple mono- and oligosaccharides binds to all three lectins with the highest affinity (Bhattacharyya et al., 1989).

Gal-1 from CHO cells and C2S-Gal-1 are dimers of molecular mass approximately 29 kDa (Cho & Cummings, 1995a). The native lectin, like most galectins, requires a reducing agent in solution to maintain its activity, while C2S-Gal-1 does not. Gal-1 possesses a $K_d \approx 7$ μM for the monomer−dimer equilibrium of its subunits (Cho & Cummings, 1995a). Both forms of the lectin have a binding preference for LacNAc and poly *N*-acetyllactosamine structures, as found for other members of the galectin family of proteins (Ahmed et al., 1990; Lee et al., 1990; Leffler & Barondes, 1986). The monomeric mutant *N*-Gal-1 shows similar binding specificity (Cho and Cummings, in press).

Thermodynamics of Saccharide Binding to SBA. The titration microcalorimetry data in Table 1 shows that SBA possesses 20- and 50-fold higher affinity constants for GalNAc and MeβGalNAc, respectively, compared to that of MeβGal, as previously reported (Lis et al., 1970; Pereira et al., 1974). Since GalNAc is a mixture of α- and β-anomers, a comparison between the two methyl β-anomers reveals that MeβGalNAc possesses a ΔH of −13.9 kcal mol^{-1} compared to −10.6 kcal mol^{-1} for MeβGal. The larger $-\Delta H$ for MeβGalNAc indicates greater binding of the acetamido group of MeβGalNAc relative to the hydroxyl group at C-2 in MeβGal. The X-ray crystal structure of SBA cross-linked with a biantennary pentasaccharide possessing terminal LacNAc residues suggest that replacement of the bound Gal moiety in LacNAc by GalNAc would allow the *N*-acetyl group of the latter to form a hydrogen bond with the side chain of Asp 88 (Dessen et al., 1995). Interestingly, although the affinity of MeβGalNAc for SBA is 3-fold greater than GalNAc, MeβGalNAc possesses −4.4 kcal mol^{-1} greater $-\Delta H$, indicating either the involvement of the β-methyl group of MeβGalNAc in binding or the negative effects of a free anomeric hydroxyl group of GalNAc. This difference in ΔH is compensated by the $T\Delta S$ term for GalNAc (−4.0 kcal mol^{-1}), which is more favorable than that for MeβGalNAc (−7.9 kcal mol^{-1}).

The $-\Delta H$ values of lactose, LacNAc, and MeβLacNAc are lower than that of MeβGal (Table 1), even though the affinities of the disaccharides are nearly the same as the monosaccharide. Hence, −ΔH for lactose is −5.1 kcal mol^{-1} less than that of MeβGal. The presence of the acetamido group in LacNAc and MeβLacNAc increases their $-\Delta H$ values relative to lactose, indicating the involvement of the acetamido group in binding. The X-ray crystal structure of SBA shows binding of the acetamido nitrogen of the GlcNAc residue of LacNAc via a hydrogen bond to Asp 215 of the lectin (Dessen et al., 1995). The X-ray crystallographic data also shows binding of the 3-, 4-, and 6-hydroxyl groups of the terminal Gal residue of LacNAc to SBA, consistent with the dominant thermodynamic role of the Gal moiety of LacNAc in binding to the lectin (Table 1).

Thermodynamics of Saccharide Binding to ECorL and ECL. ECorL and ECL possess similar thermodynamic binding data for the mono- and disaccharides shown in Table 1. For example, ECorL binds MeβGal with a ΔH of −4.4 kcal mol^{-1} and a $K_a = 0.43 \times 10^3$ M^{-1}, while ECL binds to the monosaccharide with a ΔH of −4.7 kcal mol^{-1} and a $K_a = 0.88 \times 10^3$ M^{-1}. Both ECorL and ECL possess approximately 3−4-fold higher affinities and slightly greater $-\Delta H$ values for MeβGalNAc as compared to MeβGal, indicating binding of the acetamido group of the former. The thermodynamic binding data for GalNAc and MeβGalNAc are similar for both lectins, which indicates that the β-anomeric methyl group of MeβGalNAc is not involved in binding. ECorL and ECL possess slighly higher affinities (3−4-fold, respectively) and somewhat greater $-\Delta H$ values ($\Delta\Delta H$ of −1.3 to −2.3 kcal mol^{-1}, respectively) for lactose as compared to MeβGal. The affinity constants of ECorL and EIL for LacNAc are greater (6−10-fold, respectively) than for MeβGal, and LacNAc possesses an even greater $-\Delta H$ ($\Delta\Delta H = -6.9$ and −6.2 kcal mol^{-1}, respectively) than the monosaccharide. Thus, the data indicate extended site binding interactions of the two lectins with both the Gal and GlcNAc residues of LacNAc. The thermodynamic values for LacNAc and MeβLacNAc are similar, indicating little sensitivity to binding of the β-anomeric methyl group of the latter. The same conclusion is apparent for MeβGalNAc and GalNAc.

Table 1: Thermodynamic Parameters for the Binding of Saccharides to Gal-Specific Lectins at 27 °C as Determined by Titration Microcalorimetry

carbohydrate	carbohydrate concn (mM)	lectin concn (mM)	K_a[a] (M^{-1})	ΔC_p (cal mol^{-1} K^{-1})
		SBA		
MeβGal	100	1.49	0.5×10^3 (±0.05)	−94
GalNAc	31	0.40	0.9×10^4 (±0.2)	
MeβGalNAc	30	0.54	2.2×10^4 (±0.1)	−100
lactose	132	2.80	0.2×10^3 (±0.04)	
LacNAc	100	1.57	0.7×10^3 (±0.1)	
MeβLacNAc	60	0.84	0.5×10^3 (±0.05)	
		EIL		
MeβGal	100	1.67	0.6×10^3 (±0.03)	
GalNAc	100	0.41	1.1×10^3 (±0.03)	
MeβGalNAc	95	1.35	1.6×10^3 (±0.04)	
lactose	100	0.80	3.1×10^3 (±0.1)	−200
LacNAc	21	0.34	2.1×10^4 (±0.1)	−244
MeβLacNAc	10.5	0.36	1.3×10^4 (±0.01)	
		ECorL		
MeβGal	102	2.4	0.4×10^3 (±0.07)	
GalNAc	95	0.91	1.2×10^3 (±0.2)	
MeβGalNAc	95	0.84	1.3×10^3 (±0.01)	
lactose	95	0.97	1.6×10^3 (±0.1)	
LacNAc	45	0.50	4.2×10^3 (±0.1)	94
MeβLacNAc	38	0.49	4.5×10^3 (±0.3)	
		ECL		
MeβGal	90	1.55	0.9×10^3 (±0.04)	
GalNAc	100	1.21	1.6×10^3 (±0.2)	
MeβGalNAc	50	0.72	1.4×10^3 (±0.1)	
lactose	100	0.72	3.8×10^3 (±0.2)	
LacNAc	10	0.39	1.1×10^4 (±0.2)	106
MeβLacNAc	10.5	0.59	9.9×10^3 (±0.1)	
		Gal-1		
LacNAc	11	0.33	6.2×10^3 (±0.1)	−90
dithiogalactoside	9.5	0.44	2.9×10^3(±0.2)	
		C2S-Gal-1		
LacNAc	12	0.37	2.9×10^3 (±0.3)	
dithiogalactoside	11	0.35	2.5×10^3 (±0.1)	
		N-Gal-1		
LacNAc	11	0.14	8.7×10^3 (±0.3)	

carbohydrate	$-\Delta H^a$ (kcal mol^{-1})	$-T\Delta S$ (kcal mol^{-1})	n^a (no. of sites/monomer)
	SBA		
MeβGal	10.6 (±0.4)	6.9	1.00 (±0.01)
GalNAc	9.5 (±0.1)	4.1	1.00 (±0.01)
MeβGalNAc	13.9 (±0.2)	8.0	1.00 (±0.01)
lactose	5.5 (±0.3)	2.4	1.03 (±0.03)
LacNAc	8.2 (±0.2)	4.3	1.03 (±0.03)
MeβLacNAc	7.5 (±0.4)	3.8	1.03 (±0.04)
	EIL		
MeβGal	5.9 (±0.2)	2.1	1.00 (±0.02)
GalNAc	4.9 (±0.1)	0.7	1.02 (±0.03)
MeβGalNAc	5.2 (±0.2)	0.8	1.02 (±0.03)
lactose	10.4 (±0.3)	5.6	1.02 (±0.02)
LacNAc	13.7 (±0.3)	7.8	1.02 (±0.02)
MeβLacNAc	10.4 (±0.2)	4.8	1.01 (±0.01)
	ECorL		
MeβGal	4.4 (±0.4)	0.8	1.01 (±0.02)
GalNAc	7.1(±0.4)	2.9	1.02 (±0.02)
MeβGalNAc	6.8 (±0.4)	2.5	1.04 (±0.04)
lactose	6.3 (±0.4)	1.9	1.04 (±0.04)
LacNAc	10.9 (±0.1)	5.9	1.02 (±0.02)
MeβLacNAc	11.3 (±0.3)	6.3	1.03 (±0.04)
	ECL		
MeβGal	4.7 (±0.4)	0.7	1.03 (±0.04)
GalNAc	7.3 (±0.2)	2.9	1.01 (±0.01)
MeβGalNAc	6.8 (±0.2)	2.5	1.01 (±0.01)
lactose	6.0 (±0.2)	1.1	1.00 (±0.01)
LacNAc	10.9 (±0.1)	5.4	1.03 (±0.03)
MeβLacNAc	10.3 (±0.4)	4.8	1.00 (±0.01)
	Gal-1		
LacNAc	6.6 (±0.3)	1.4	1.03 (±0.02)
dithiogalactoside	3.8 (±0.1)	−0.94	1.02 (±0.02)
	C2S-Gal-1		
LacNAc	2.8 (±0.3)	−1.94	1.04 (±0.04)
dithiogalactoside	2.6 (±0.1)	−2.05	1.02 (±0.02)
	N-Gal-1		
LacNAc	0.6 (±0.3)	−4.79	1.00 (±0.02)

The association constants and $-\Delta H$ values reported by De Boeck et al. (1984b) for ECL using a competitive binding assay with *N*-dansylgalactosamine as a fluorescent indicator ligand are similar for GalNAc in the present study, but their $-\Delta H$ values are higher than those for lactose and LacNAc obtained by titration microcalorimetry in the present study.

The X-ray crystal structure of lactose bound to ECorL has been determined at 2 Å resolution (Shaanan et al., 1991). The structure shows that the combining site of ECorL is a shallow depression on the protein surface that binds to the 3-, 4-, and 6-hydroxyls of the Gal moiety of lactose. An open space in the binding site was observed close to the 2-OH of the bound Gal residue which could accommodate the acetamido group of GalNAc, as suggested by the thermodynamic data. The crystal structure also shows that the Glc residue of lactose resides mostly outside the binding pocket and is poorly defined in the electron density map, indicating its flexibility in the bound complex and lack of strong interactions. This is in agreement with the relatively small differences in the thermodynamic data for MeβGal and lactose (Table 1). However, the thermodynamic data also indicate that the GlcNAc moiety of LacNAc contributes to binding. Therefore, it will be important to co-crystallize LacNAc with ECorL to determine if this interaction can be observed in the crystalline complex. In any case, the data in Table 1 indicate that ECorL and ECL possess very similar thermodynamic mechanisms of binding carbohydrates, and hence their combining site topologies must be very similar.

Thermodynamics of Saccharide Binding to EIL. EIL exhibits somewhat different thermodynamic binding data relative to ECorL and EIL in Table 1. While ECorL and EIL show enhanced $-\Delta H$ values for GalNAc and MeβLacNAc relative to MeβGal, EIL demonstrates little change in $-\Delta H$ for all three monosaccharides. Furthermore, EIL exhibits $-\Delta H$ values for lactose and LacNAc of -10.4 and -13.7 kcal mol^{-1}, respectively, as compared to -6.3 and -10.9 kcal mol^{-1}, respectively, for ECorL and -6.0 and -10.9 kcal mol^{-1}, respectively, for ECL. However, the affinity of lactose for all three lectins is nearly the same, as is the case with LacNAc, even though the $-\Delta H$ values of EIL for lactose and LacNAc are greater than the other two lectins. Previous equilibrium dialysis studies with EIL and lactose resulted in a $K_a = 2.2 \times 10^3$ M^{-1} at 25 °C and a van't Hoff ΔH value of -9.4 kcal mol^{-1} and $T\Delta S$ of -4.9 kcal mol^{-1} (Bhattacharyya et al., 1981), which are values consistent with the present results.

These results indicate that EIL, although a member of the *Erythrina* lectin family with similar binding specificity, possesses somewhat different thermodynamic mechanisms for binding carbohydrates as compared to ECorL and EIL. Interestingly, the molecular mass (68 kDa) of EIL as well as its extinction coefficient of 13.4 at 280 nm is different as compared to that of ECL and ECorL which have molecular masses of 56 kDa and extinction coefficients of 15.3 and 15.9, respectively, at 280 nm (Goldstein & Poretz, 1986).

Thermodynamics of LacNAc Binding to Gal-1 from CHO Cells and Two Mutants. Gal-1 from CHO cells and C2S-Gal-1 have recently been expressed as recombinant proteins in *E. coli* (Cho & Cummings, 1995a). Biosynthetic studies reveal that the lectin is secreted by CHO cells and is found both at the cell surface, where it is bound to surface glycoconjugates, and in the media in free form (Cho & Cummings, 1995b). C2S-Gal-1 was constructed as a stable form of the lectin (Cho & Cummings, 1995a), because the six cysteine residues that are found in galectins as free sulfhydryls, although not critical for activity, contribute to the instability of the lectins in the absence of reducing agents (Abbott & Feizi, 1991; Hirabayashi & Kasai, 1991). C2S-Gal-1 was demonstrated to be more stable than the native lectin in solutions lacking reducing agents (Cho & Cummings, 1995a), thus providing evidence that Cys-2 is responsible for the observed instability of the lectin. Recently, a monomeric mutant of the lectin (*N*-Gal-1), possessing the C2S mutation as well as three other amino acid changes in the N-terminal region, was expressed in *E. coli* and shown to have similar binding activity (Cho and Cummings, in press).

Table 1 shows the thermodynamic data for binding of Gal-1 and C2S-Gal-1 to LacNAc and dithiogalactoside. Similar data is presented for binding of *N*-Gal-1 to LacNAc. The K_a values of Gal-1, C2S-Gal-1, and *N*-Gal-1 for LacNAc are nearly the same: 6.2×10^3, 2.9×10^3, and 8.7×10^3 M^{-1}, respectively. These results agree well with equilibium dialysis experiments that demonstrate that all three proteins possess K_d values of $\sim 1 \times 10^{-4}$ M for LacNAc (Cho and Cummings, in press). The data are also consistent with the observation that Gal-1 and C2S-Gal-1 bind to immobilized laminin with essentially equal avidities (Cho & Cummings, 1995a). Importantly, the ΔH and $T\Delta S$ values of the three forms of the lectin binding to LacNAc are substantially different. For Gal-1, ΔH is -6.6 kcal mol^{-1}, while for C2S-Gal-1 and *N*-Gal-1 the ΔH values are -2.8 and -0.6 kcal mol^{-1}, respectively. Furthermore, K_a values of Gal-1 and C2S-Gal-1 for dithiogalactoside are also nearly the same, 2.9×10^3 and 2.5×10^3 M^{-1}, respectively. However, ΔH for the native lectin is -3.8 kcal mol^{-1} while ΔH for the C2S mutant is -2.7 kcal mol^{-1}. Thus, the differences in ΔH (and $T\Delta S$) values for Gal-1, C2S-Gal-1, and *N*-Gal-1 are due to intrinsic differences in the proteins and not to the individual saccharides.

The position of Cys-2 in the X-ray structures of bovine spleen Gal-1 as well as human galectin-2 (Lobsanov et al., 1993) is far removed (approximately 20 Å) from the carbohydrate binding sites of the lectins. Thus, the differences in $-\Delta H$ values for binding LacNAc and dithiogalactoside to Gal-1 (-6.6 and -3.8 kcal mol^{-1}, respectively) as compared to C2S-Gal-1 (-3.8 and -2.6 kcal mol^{-1}, respectively) are due to long-range effects and not to contact residues in the binding site. Changes in the N-terminal region of *N*-Gal-1 (residues 2, 4, 5, and 6) are likewise far removed from the carbohydrate binding site of the lectin. The same must be true for the differences in the $-\Delta H$ value (-0.6 kcal mol^{-1}) of *N*-Gal-1 binding LacNAc as compared to the other two lectins. Similar long-range changes in ΔH (and $T\Delta S$) without changes in ΔG have recently been observed in mutants of glucoamylase from *Aspergillus niger* (Berland et al., 1995). It will thus be important to determined the molecular mechanisms that give rise to the differences in ΔH between native Gal-1 and the C2S mutant in order to understand the thermodynamic binding parameters of the lectin. It is interesting to note that the ΔH and $T\Delta S$ values for Gal-1, C2S-Gal-1, and *N*-Gal-1 binding to LacNAc all fall on the same line (Figure 3).

The crystal structure of Gal-1 from bovine spleen complexed with LacNAc, determined at 1.9 Å resolution (Liao et al., 1994), shows that the 4- and 6-hydroxyl groups and the ring oxygen atom of the Gal residue and the 3-hydroxyls of the GlcNAc residue of LacNAc are directly involved in

Table 2: Thermodynamic Parameters of the Binding of the Gal-Specific Lectins to the Carbohydrates

parameters[a]	T (°C)	SBA-MeβGal	SBA-MeβGalNAc	Gal-1-LacNAc	EIL-lactose	EIL-LacNAc	ECL-LacNAc	ECorL-LacNAc
K_a	9	1.3×10^3	64×10^3	1.74×10^3	4.74×10^3	34×10^3	74×10^3	31×10^3
$-\Delta H$	9	8.9	12.1	5.5	7.4	10.9	12.8	12.6
ΔS_u	9	−9.4	−12.9	+3.4	−1.6	−10.1	−15.1	−16.1
$-\Delta G_u$	9	6.3	8.5	6.5	7.0	8.1	8.6	8.1
K_a	27	0.45×10^3	22×10^3	6.2×10^3	3.1×10^3	21×10^3	11×10^3	4.2×10^3
$-\Delta H$	27	10.6	13.9	6.6	10.4	13.7	10.9	10.9
ΔS_u	27	−15.4	−18.7	+3.3	−10.7	−18.0	−10.0	−11.7
$-\Delta G_u$	27	6.0	8.3	7.6	7.2	8.3	7.9	7.4
ΔC_p		−94	−100	−90	−200	−244	+106	+94

[a] Units: K, M^{-1}; $\Delta H°$ and $\Delta G°$, kcal/mol; ΔS_u, cal/mol deg (eu); ΔC_p, cal/mol deg. The unitary entropy (ΔS_u) and unitary free energy (ΔG_u) are determined from $\Delta S°$ and $\Delta G°$ as follows: $\Delta S_u = \Delta S° + 7.98$ eu; $\Delta G_u = \Delta G° - 0.00798T$ kcal/mol.

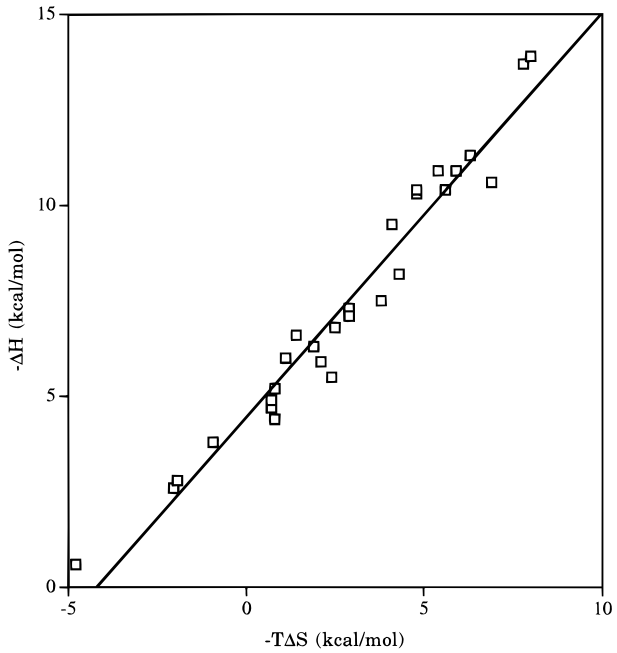

FIGURE 3: Plot of $-\Delta H$ versus $-T\Delta S$ data in Table 1 for the binding of the carbohydrates in the table to SBA, EIL, ECL, ECorL, CHO Gal-1, C2S-Gal-1, and *N*-Gal-1.

hydrogen binding to the protein. These interactions are likely to be conserved in Gal-1 from CHO cells, since its primary structrure is 95% identical to bovine Gal-1, and to contribute to the observed thermodynamic parameters.

Interestingly, the $-\Delta H$ value for LacNAc binding to the native 14 kDa lectin is lower than those values for the plant lectins, even though their K_a values are of the same order of magnitude. Thus, the animal lectin possesses a greater contribution of $T\Delta S$ of binding of the disaccharide than the plant lectins, indicating a different thermodynamic mechanism of binding of the animal lectin. In addition, C2S-Gal-1 and *N*-Gal-1 possess even greater contributions of $T\Delta S$ to binding of the disaccharide than Gal-1.

Surolia and co-workers have reported a ΔH value of -10 kcal mol^{-1} for the binding of LacNAc to galactin-1 from sheep spleen (Ramkumar et al., 1995). This may indicate that galectin-1 from different sources may possess different binding energetics based on differences in their primary structures.

Enthalpy−Entropy Compensation. Changes in ΔH and $T\Delta S$ upon binding of mono- and disaccharides to SBA, EIL, ECL, ECorL, Gal-1, C2S-Gal-1, and *N*-Gal-1 are compensatory, as shown in Figure 3. The plot shows a linear relationship with a slope of 1.07 (correlation coefficient of 0.931). Changes in ΔH and $T\Delta S$ upon binding of mono- and disaccharides to SBA, EIL, ECL, and ECorL were also plotted separately for each lectin (not shown). The plots show slopes of 1.28 (correlation coefficient of 0.897) for SBA, 1.20 (correlation coefficient of 0.977) for ECL, 1.27 (correlation coefficient of 0.987) for EIL, and 1.10 (correlation coefficient of 0.989) for ECorL. The ΔH and $T\Delta S$ values for native Gal-1, C2S-Gal-1, and *N*-Gal-1 binding to LacNAc are also shown in Figure 3. Their values fall on the same general slope that describes the ΔH versus $T\Delta S$ values for the Gal-specific plant lectins binding to mono- and disaccharides. Such enthalpy−entropy compensations have been previously observed for the interaction of carbohydrates with lectins (Lemieux et al., 1991; Schwarz et al., 1993) and antibody (Brummell et al., 1993; Herrons et al., 1986; Sigurskjold & Bundle, 1992), and attributed to the unique properties of water (Lemieux et al., 1991). These compensations have also been demonstrated in the binding of carbohydrates of increasing size to lectins (Hindsgaul et al., 1985) and for the substitution of hydroxyl group by hydrogen, fluorine, and chlorine (Spohr et al., 1992). Entropy−enthalpy compensation is also observed as a function of temperature (Fukuda et al., 1983).

The thermodynamics binding parameters in Table 1 are similar to those reported earlier for other lectin−carbohydrate interactions in that the slopes of the enthalpy−entropy plots in Figure 3 are greater than unity (Lemieux, 1989; Munske et al., 1984). This is in contrast to antibody−carbohydrate interactions, where slopes of less than unity have been reported (Brummell et al., 1993; Sigurskjold & Bundle, 1992). A slope greater than unity means that the free energy of binding is more sensitive to the enthalpy of binding, while a slope less than unity indicates a greater sensitivity to changes in entropy. It is interesting to note that the slopes and correlation coefficients of the plots for all the lectins are very similar, indicating a common mechanism of entropy−enthalpy compensation for plant and animal lectins with similar binding specificities.

Heat Capacities of Binding of the Lectins. The thermodynamics parameters of binding of lactose and LacNAc with the Gal binding lectins are summarized in Table 2. Results at two different temperatures (9 and 27 °C) give an estimate of ΔC_p, the heat capacity of binding for the lectins. ΔC_p values for SBA binding to MeβGal and MeβGalNAc are -94 and -100 cal K^{-1} mol^{-1}, respectively. ΔC_p values for EIL binding to lactose and MeβLacNAc are -200 and -244, which are also negative. ΔC_p for C2S-Gal-1 binding to LacNAc is -90 cal K^{-1} mol^{-1}. The ΔC_p values of ECL and ECorL for LacNAc are $+106$ and $+94$ cal K^{-1} mol^{-1}.

A feature of the data presented in Table 2 is the essential temperature independence of ΔG_u, the unitary free energy

of binding, which has also been observed in monoclonal antidextran IgM−oligosaccharide interactions (Zidovetzki et al., 1988). In contrast, ΔH and ΔS_u exhibit temperature dependence. Though data were not collected over a wide range of temperature, the values of ΔC_p are negative for SBA, EIL, and Gal-1 and positive for ECL and ECorL. These values are relatively small in comparison with wide variety of other binding processes involving proteins (Sturtevant, 1977).

Binding of carbohydrates to a number of proteins is characterized by small enthalpy and heat capacity changes (Dani et al., 1981; Munske et al., 1984). Hydrogen bonding interactions are essentially enthalpically driven with little change in the heat capacity, while hydrophobic interactions are essentially entropically driven (Schwarz et al., 1991). In many cases, however, binding of saccharides is coupled to changes in solvent accessibilities that result in negative, albeit small, ΔC_p values (Bains et al., 1992).

Summary. The present results demonstrate the thermodynamic flexibility of carbohydrate binding to Gal-specific plant and animal lectins. Three members of the *Erythrina* lectin family, EIL, ECorL, and ECL, have similar K_a values for lactose and LacNAc; however, EIL possesses greater $-\Delta H$ values for the disaccharides. SBA, on the other hand, shows a completely different thermodynamic pattern of binding mono- and disaccharides as compared to the *Erythrina* lectins. Gal-1 from CHO cells possesses a K_a value for LacNAc which is similar to the K_a values for the *Erythrina* lectins, however, the animal lectin exhibits a smaller $-\Delta H$ value for binding the disaccharide. In addition, two mutants of Gal-1 exhibit similar K_a values for LacNAc as that of the native lectin, but the mutants possess much lower $-\Delta H$ values for the disaccharide even though the sites of mutation of the lectin are far removed from the carbohydrate binding site(s). Interestingly, an entropy−enthalpy compensation plot shows that all of the plant and animal lectin data fall on the same slope, which indicates a general thermodynamic compensation mechanism shared by the proteins. Thus, the present study reveals the wide range of thermodynamic mechanisms exhibited by Gal-specific plant and animal lectins. A key question, therefore, is to relate the structures of the lectins, where known, to their thermodynamic binding paramerters. This is especially intriguing in the case of mutations away from the binding site of the lectin that effect the thermodynamic binding paramerters, as with Gal-1 and its two mutants in the present study.

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