Calorimetric Analysis of the Binding of Lectins with Overlapping Carbohydrate-Binding Ligand Specificities†

Mary C. Chervenak and Eric J. Toone*

Department of Chemistry, Duke University, Durham, North Carolina 27708-0346

Received October 14, 1994; Revised Manuscript Received January 18, 1995⊗

ABSTRACT: The thermodynamics of binding of a system of plant lectins specific for the oligosaccharide methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside have been studied calorimetrically. This system of lectins consists of concanavalin A, the lectin isolated from *Dioclea grandiflora*, and the lectin from *Galanthus nivalis*. The group thus contains lectins with similar structures and similar binding properties as well as lectins with different structures but similar binding properties. Concanavalin A and the lectin from *Dioclea* are highly homologous, while the lectin from *Galanthus nivalis* shares no sequence homology with either of the legume lectins, although it also binds the mannose trisaccharide tightly. Calorimetric data for oligosaccharide binding to both of the legume lectins suggests that the total binding site comprises a single high-affinity site and an additional extended site. The pattern of binding for the lectin from *Galanthus* is significantly different. Binding studies with the same saccharides indicate that the lectin has binding sites designed specifically for the 1→3 and 1→6 arms of the mannose trisaccharide that are unable to accommodate other saccharides. Enthalpy−entropy compensation was observed for several saccharides as a function of lectin structure. Contributions of solvation effects to the enthalpy of binding and the configurational entropies were determined experimentally. For those systems studied here, solute−solute attractive interactions and configurational entropies were the greatest contributors to enthalpy−entropy compensation. Our studies clearly demonstrate that, despite their common affinity for the mannose trisaccharide, the three lectins bind oligosaccharides very differently.

In vivo, carbohydrates act as intermediates of biological communication (Grant & Peters, 1984; Lis & Sharon, 1986; Feizi & Childs, 1987; Spiess, 1990; Takeichi, 1991; Varki, 1993). The information contained in the sophisticated array of oligosaccharides on cell surfaces is recognized by a specialized group of proteins, the lectins. Carbohydrate−lectin interactions control many facets of cell life, including growth and differentiation, cell aggregation and migration, and cellular development (Lis & Sharon, 1986; Feizi & Childs, 1987; Sharon & Lis, 1989; Berels *et al.*, 1991; Drickamer & Taylor, 1993). Protein−carbohydrate interaction is also a controlling step in myriad human disease states. Specifically, many parasitic, bacterial, and viral pathogens recognize and adhere to a host cell surface through protein−carbohydrate interaction (Karlsson, 1989; Manocha & Chen, 1989; Anderson *et al.*, 1991; Ingram & Molyneux, 1991). Furthermore, the nonrandom distribution of malignancies is thought to involve carbohydrate-based recognition (Liener *et al.*, 1986; Itzkowitz *et al.*, 1990; Gabius & Gabius, 1991; Hiraizumi *et al.*, 1992; Muramatsu, 1993). Because of the roles of protein−carbohydrate interaction in human disease, the development of carbohydrate-based therapeutics designed to interrupt biological recognition is an area of intense research. Such mimics would represent a fundamentally new class of therapeutic agents, designed to act by disrupting biological communication, instead of as cytotoxins.

A daunting barrier to the development of therapeutic carbohydrates is the need to ensure *specificity* during lectin−ligand interaction. Although carbohydrate-mediated recognition is ubiquitous in both intra- and intercellular communication, only eight monosaccharides are transferred in mammalian biochemistry (Lis & Sharon, 1986). Consequently, *in vivo*, multiple lectins exist with overlapping carbohydrate binding specificities. To investigate the feasibility of constructing a carbohydrate that would bind specifically to a single lectin in the presence of multiple lectins with overlapping or identical binding specificities, we have exploited a model system of plant lectins that share an affinity for the trisaccharide methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside, **17** (Figure 1). This trimannoside represents the core of all N-linked mammalian glycoproteins.

Two categories of lectins with overlapping specificities will be encountered *in vivo*; lectins with overlapping binding specificity and *extensive* structural homology and lectins with overlapping binding specificity but *little or no* structural homology. We have constructed our model to address both eventualities. The first member of the group, the lectin from *Canavalia ensiformis*, or concanavalin A (con A), is the best studied of all plant lectins (Liener, 1976). Originally purified by Sumner and Howell in 1936, it was identified as a mannose/glucose-specific lectin. In 1985, Brewer and coworkers identified the mannose trisaccharide as the minimum epitope that completely fills the carbohyrate binding site (Brewer *et al.*, 1985). In solution, concanavalin A exists as a dimer below pH 5.2 and as a tetramer above pH 7.0. Each identical subunit binds one saccharide and two metal ions, both of which are necessary for carbohydrate

† This work was supported in part by a grant from the National Institutes of Health (GM48653). M.C.C. acknowledges the Pharmacological Sciences Training Program (2T32GM07105) at Duke University for stipend support. E.J.T. was a 1992−1993 American Cyanamid Faculty Fellow.

⊗ Abstract published in *Advance ACS Abstracts,* April 1, 1995.

0006-2960/95/0434-5685$09.00/0

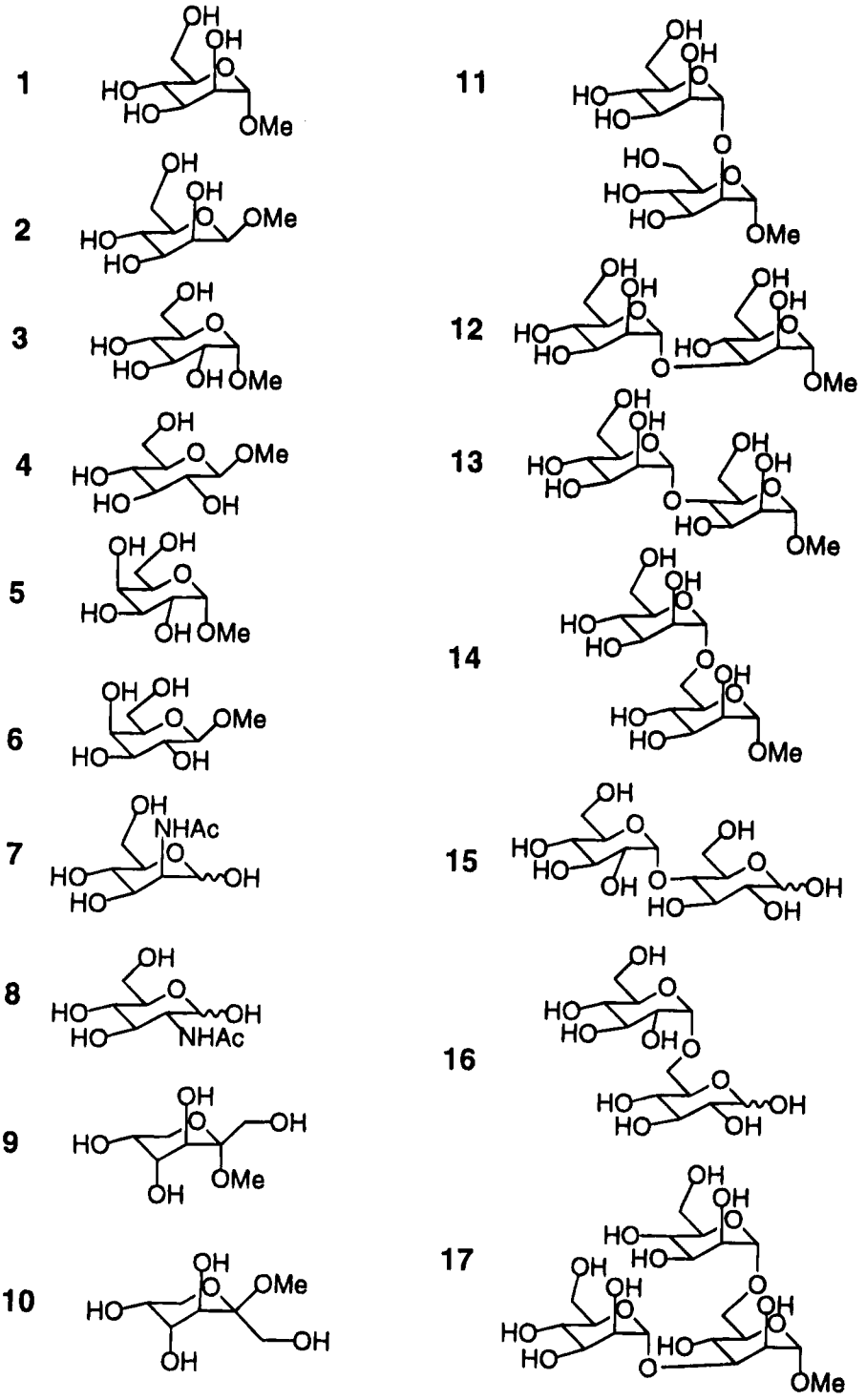

FIGURE 1: Structures of mono- (**1**−**10**) and oligosaccharide (**11**−**17**) lectin ligands.

binding. The oligosaccharide binding site is a shallow pocket located about 8.7 Å from the Ca^{2+} binding site and 12.8 Å from the transition metal binding site (Derewenda *et al.*, 1989).

In 1983, Moreira and co-workers reported the isolation of a lectin from *Dioclea grandiflora*, a legume indigenous to northeastern Brazil: this lectin is the second member of our group. The *Dioclea grandiflora* lectin also belongs to the general group of mannose/glucose-specific legume lectins. Unlike concanavalin A, *Dioclea* is a tetramer at all pH levels, with a subunit molecular weight of 26 000 (Moreira *et al.*, 1983). Little is known about the carbohydrate binding properties of the lectin. This protein is very nearly a mutant of concanavalan A; their sequences differ in only 59 of 237 residues, with no gaps (Richardson *et al.*, 1984). Both plants belong to the tribe *Diocleae*, and this is likely the source of their homology.

The snowdrop (*Galanthus nivalis*) lectin, the final member of our group, is a mannose-binding lectin present in the tubers of the family Amaryllidaceae. First isolated by Van Damme and co-workers in 1987, the lectin is a tetramer at pH 7.0 and has a monomeric weight of 15 000 (Van Damme *et al.*, 1987). The lectin is of interest here because it is specific for the trimannoside, but shows little or no sequence homology to any of the legume lectins. We present here our initial findings on the similarities and differences in

saccharide binding of the lectins from *Canavalia ensiformis*, *Dioclea grandiflora*, and *Galanthus nivalis*.

MATERIALS AND METHODS

General. Buffer salts were purchased from Aldrich Chemical company and used without further purification. Water in all cases was purified with a Millipore purification system that involved passage through reverse osmosis, charcoal, and two ion-exchange filters. The water had a resistance of >10 MΩ cm^{-1}.

Carbohydrates. Isomaltose (lot 72H4073), maltose (lot 113H0567), methyl α-D-galactopyranoside (lot 108F5038), methyl β-D-galactopyranoside (lot 72H5026), methyl β-D-glucopyranoside (lot 93H3865), methyl β-D-mannopyranoside (lot 32H4047), *N*-acetyl-D-glucosamine (lot 43H0432), and *N*-acetyl-β-D-mannosamine (lot 63H5006) were purchased from Sigma Chemical company and used without further purification. Methyl α-D-glucopyranoside (lot 05508EW) and methyl α-D-mannopyranoside (lot 10312DW) were purchased from Aldrich Chemical company and used without further purification. Methyl 2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside, methyl 3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside, methyl 4-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside, methyl 6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside, methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside, methyl α-D-fructopyranoside, and methyl β-D-fructopyranoside were synthesized using modifications of literature procedures; details of syntheses will be reported elsewhere. In each case, carbohydrate ligands were >95% pure by NMR. Carbohydrate concentrations were determined by phenol−sulfuric acid chars according to the method of Dubois (1956).

Protein Purification. Concanavalin A (Type IV, lot 81H7020) was either purchased from Sigma Chemical company and used without further purification or isolated from commercial jack bean meal (Sigma) by affinity chromatography on Sephadex G75, according to literature procedures (Agrawal & Goldstein, 1967). The lectin from *Dioclea grandiflora* was purified from seed samples collected in northeastern Brazil by affinity chromatography on Sephadex G75 according to literature procedures (Moreira *et al.*, 1983). The lectin from *Galanthus nivalis* (Burpee Seed Company) was purified by affinity chromatography using a D-mannose functionalized cross-linked agarose resin (Sigma), according to literature procedures (Van Damme *et al.*, 1987). The lectin from *Dioclea grandiflora* and snowdrop lectin gave single-band Comassie-stained SDS-PAGE gels. Concanavalin A gave bands at 26 KDa (monomer) as well as at 12 and 14 KDa, representing previously described nicked fragments (Cunningham *et al.*, 1972). Protein concentrations for concanavalin A and the lectin from *Dioclea grandiflora* were determined by the method of Edelhoch (1967). Concentrations for the snowdrop lectin were measured spectrophotometrically, using the extinction coefficient of Goldstein (Kaku & Goldstein, 1989).

Calorimetry. Calorimetric measurements were made using the Microcal Omega titration microcalorimeter; details of instrument design and data analysis are described elsewhere (Wiseman *et al.*, 1989). The calorimeter cell held a volume of 1.3678 mL. For all titrations, the lectin was placed in the cell and titrated with saccharide ligand. Lectin concentrations ranged from 300 μM to 1 mM. In all cases, the

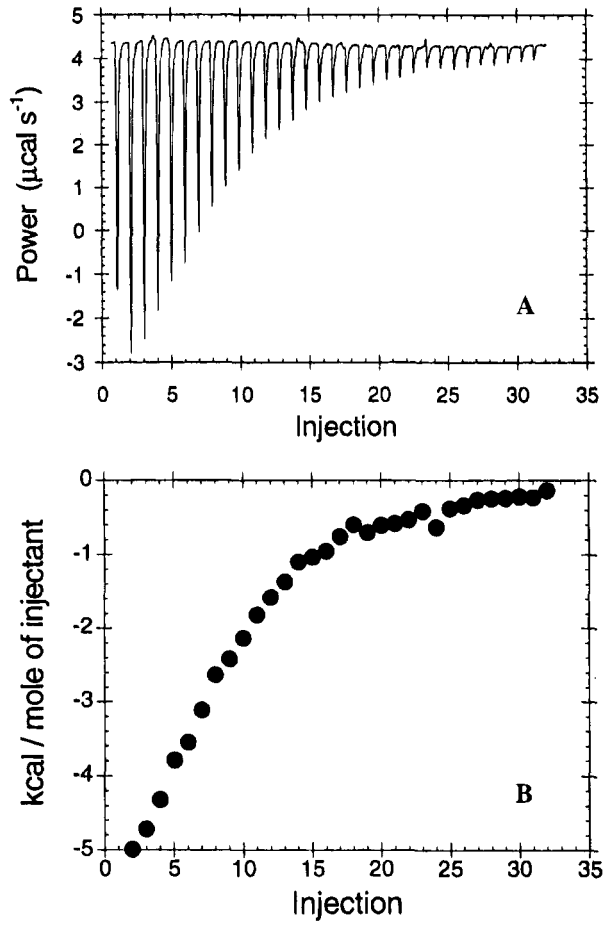

FIGURE 2: Calorimetric titration of native *Dioclea grandiflora* (0.3107 mM) with methyl 6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside **14** (14.6 mM) at 15.0 °C: (A) raw data for 40 2.2-μL injections of **14**, and (B) the integrated curve showing experimental points (•) and the least squares fit (—) to the integrated data. The buffer was 50 mM Na_2HPO_4 containing 250 mM NaCl, 1 mM $MnCl_2$, and 1 mM $CaCl_2$ at pH 7.15.

value *C*, defined as the product of the binding constant *K* and the concentration of binding sites, was in the range of 1−100, and ligand concentrations were such that the final ligand concentration was at least $10K_D$. Titrations of concanavalin A, the lectin from *Dioclea grandiflora*, or the snowdrop lectin were carried out at pH 6.92 in 50 mM phosphate buffer augmented with 500 mM NaCl and 1 mM each of $MnCl_2$ and $CaCl_2$. All protein samples were dissolved in identical buffer that included both Mn^{2+} and Ca^{2+}, although the metals were not necessary for the snowdrop lectin binding. To a solution of lectin was added ligand in 2.2-μL injections. Typically, 40 injections 4.4 s in duration were made with 3-min intervals between injections. Each injection generated a measurable heat resulting from the association of protein and ligand. The data were integrated to provide a titration curve, and by use of a nonlinear least squares fit, the binding constant, *K*, the heat of binding, ΔH, and the stoichiometry of binding were extracted from the curve. A typical data set is shown in Figure 2. Non-negligible heats of dilution were subtracted prior to data reduction.

Constant pressure heat capacities, ΔC_p, were determined by evaluating ΔH as a function of *T*. Heat capacity is rigorously defined as the partial derivative of ΔH with respect to temperature at constant pressure, *i.e.*, $\Delta C_p = (\partial H/\partial T)_P$.

Over short temperature ranges, ΔC_p is predicted to be linear, and can be approximated as $\Delta(\Delta H)/\Delta T$, or $(\Delta H_1 - \Delta H_2)/(T_1 - T_2)$.

RESULTS AND DISCUSSION

Thermodynamics of Binding of Concanavalin A with Various Oligosaccharides. Computer-assisted titration calorimetry, as described by Wiseman *et al.* (1989), is a rapid and accurate method for characterizing biological binding reactions. Titration microcalorimetry allows for a precision and conservation of biological materials not obtained with other methods. Microcalorimetry provides direct estimates of the enthalpy of binding, as well as the binding constant. Additionally, calorimetry is the only experimental technique that provides a direct measurement of ΔC_p. We show here and elsewhere that ΔC_p is vital for the molecular attribution of bulk thermodynamic properties.

The energetics of concanavalin A complexation with various oligosaccharides has previously been studied by titration microcalorimetry at pH 5.2, where concanavalin A exists as a dimer (Williams *et al.*, 1992). In this study, we have carried out calorimetry at pH 7.0, where the lectin exists as a tetramer, and have observed no significant change in the thermodynamic parameters of complexation, although enthalpies of binding are somewhat higher. Several different calorimetric values of ΔH have been reported for the interaction of concanavalin A with oligosaccharides (Ambrosino *et al.*, 1987; Williams *et al.*, 1992; Schwarz *et al.*, 1993; Mandal *et al.*, 1994). The origin of these discrepancies is clearly a cause for concern. Although seldom are the same carbohydrates investigated, there exist several independent measures of the enthalpy of binding of methyl α-D-mannopyranoside to concanavalin A. This data set makes a useful comparison group for the elucidation of experimental differences that may produce the variety of binding enthalpies that have been reported. Brewer and co-workers have suggested that the differences could be a result of different methods of ligand concentration determination; this group has recently published a modification of the original Dubois method for oligosaccharide concentration determination (Mandal *et al.*, 1994; Saha & Brewer, 1994). Although enthalpies of binding vary linearly as the ligand concentration, differences in saccharide concentration are not likely the source of differences in reported enthalpies of concanavalin A/methyl α-D-mannopyranoside binding. In calorimetric titrations, *both* ΔH and K_{eq} vary linearly as the ligand concentration. Thus, if differences in enthalpies of binding arose from differences in ligand concentrations, these differences would necessarily be reflected in equilibrium binding constants. These deviations are not observed: a plot of K_{eq} *versus* ΔH for the four reported values gives a correlation coefficient of only 0.7. Alternatively, a plot of enthalpy of binding *versus* total ionic strength (Figure 3) shows strong linear correlation ($r = 0.987$), suggesting that the enthalpy of binding of concanavalin A to oligosaccharides depends strongly on the experimental conditions used. A salt bridge, formed in the unliganded state between the side chains of Asp16 and Arg288, may be partially responsible for these differences in enthalpy; this bridge must be broken to accommodate the saccharide. As the ionic strength of the buffer in which the reaction is being run increases, the salt bridge would gradually disappear, and the positive enthalpy resulting from cleavage of the bridge during binding would be reduced. We caution, of course, against drawing strong conclusions based on a limited data set. Furthermore, a single measurement reported by Brewer and co-workers lies above the line described in Figure 3 (Mandal *et al.*, 1994).

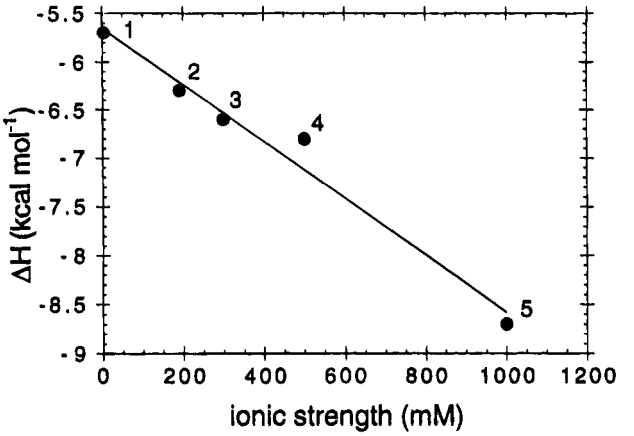

FIGURE 3: Plot of ΔH *versus* ionic strength for concanavalin A/methyl α-D-mannopyranoside interaction at 25 °C: (1) Ambrosino *et al.* (1987), (2) Schwarz *et al.* (1993), (3) Williams *et al.* (1992), (4) this work, and (5) Mandal *et al* (1994).

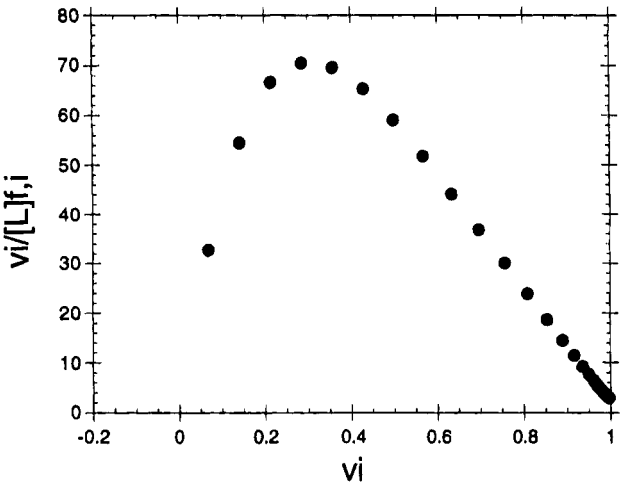

FIGURE 4: Scatchard plots for the binding of concanavalin A to methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside at pH 5.2 and 25 °C.

As we have previously noted, Scatchard plots generated from calorimetric data for concanavalin A/trimannoside interaction show slight concave-down deviation from linearity, indicative of positive cooperativity (Williams *et al.*, 1992). Recently, Brewer and co-workers created Scatchard plots for concanavalin A/trimannoside binding and reported no cooperativity (Mandal *et al.*, 1994), further reporting that their results were in agreement with those of Mackenzie (1986). Brewer and co-workers suggest that the origin of the curvature in Scatchard plots of tight-binding ligands is errors in the evolved enthalpies in the first few points. We consistently generate Scatchard plots of the shape shown in Figure 4, and we note that this plot is in complete accord with those generated by Brewer and co-workers; only the meaning of the curvature is in question. In the work cited by Brewer, strong curvature was observed in *all* Scatchard plots (Figure 5). The magnitude of the curvature was temperature dependent, decreasing with increasing temperature. We observe similar behavior for both concanavalin A and the *Dioclea* lectin (*vide infra*).

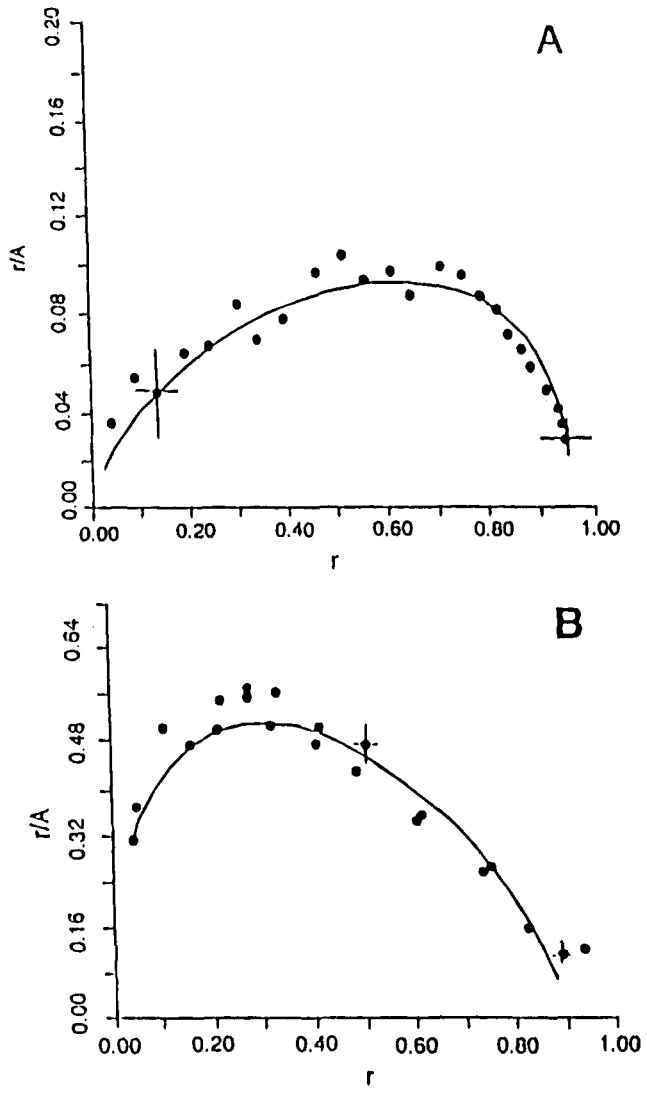

FIGURE 5: Scatchard plots for the binding of concanavalin A to methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside [from Mackenzie (1986); reproduced with permission]: (A) plot generated from fluorescence data at pH 5.4 and 20.5 °C and (B) plot generated from equilibrium dialysis data at pH 5.4 and 17.4 °C (equilibration time, 13 h).

Cooperative binding can arise from a variety of mechanisms. Allosteric changes in protein conformation that occur during the binding of the first ligand can allow a more favorable second binding. Alternatively, cooperativity may arise from dynamic relaxation of the binding site. It has long been recognized that conformational shifts accompanying binding essentially provide a binding site with a low- and a high-affinity state. If the rate of ligand binding becomes fast relative to relaxation of the binding site back to its low-affinity state following dissociation, the binding will appear cooperative (Figure 6). By definition K_3 (*ie.*, k_3/k_{-3}) > K_2; if $k_3 > k_{-1}$ and $k_{-2} \sim k_4$, then as the concentration of ligand L increases, the binding will appear cooperative as the concentration of high-affinity binding sites increases in solution. If this latter mechanism is the source of cooperativity, the curvature of the Scatchard plots should be temperature dependent: we observe temperature-dependent curvature, and this behavior has been reported by others (Mackenzie, 1986). This "kinetic" origin of cooperativity for concanavalin A binding has been suggested previously

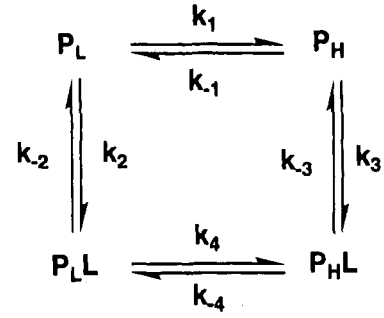

FIGURE 6: Schematic representation of binding and reorganization of a lectin binding site.

(Mackenzie, 1986). The phenomenon has also been reviewed (Citri, 1973; Weber, 1975).

The results of our work, Mackenzie's work, and, we submit, Brewer's work convincingly demonstrate positive cooperativity in concanavalin A/trimannoside binding. Furthermore, Brewer's Scatchard plot of succinyl concanavalin A/trimannoside interaction shows significantly less curvature than does the plot for native concanavalin A. This observation may be interesting in light of our hypothesis of the origin of cooperativity in concanavalin A binding.

Thermodynamic parameters for the interaction of concanavalin A with the α- and β-anomers of methyl mannopyranoside, methyl glucopyranoside, methyl galactopyranoside, and methyl fructopyranoside as well as with 2-deoxy-2-acetamidoglucopyranoside, 2-deoxy-2-acetamidomannopyranoside, disaccharides **11**–**16**, and trisaccaride **17** are summarized in Table 1. The corresponding binding constants and the concentrations of lectin and carbohydrate used in their determination in shown in Table 2. The relative magnitudes of the binding constants and the enthalpies of binding are in good accord with the results of Mandal *et al.*, although our enthalpies are uniformly smaller. The thermodynamics of binding are consistent with a single high-affinity binding site that binds the nonreducing termini of all disaccharides, and an extended site that interacts with both termini of the trisaccharide: this motif has previously been identified. The requirements for binding within the high-affinity site have been well described (Goldstein *et al.*, 1965; Carver *et al.*, 1985; Derewenda *et al.*, 1989; Mandal *et al.*, 1994), and our data are in accord with these observations.

Enthalpy–entropy compensation as a function of ligand structure is again observed (Eftink & Biltonen, 1980). For example, methyl α-D-glucopyranoside (**3**), maltose (**15**), and isomaltose (**16**) have similar binding constants, although enthalpies of binding vary by as much as 1.6 kcal mol^{-1}. The origin and meaning of enthalpy–entropy compensation remain a topic of debate (*vide infra*). The disaccharide methyl 2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**11**) shows an enthalpy similar to that of methyl 3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**12**), but a 3.5-fold enhanced affinity. The minimum requirements for concanavalin A binding are free equatorial hydroxyl groups at positions 3, 4, and 6. Presumably, both reducing and nonreducing termini of the 1→2 dimannoside can bind in the high-affinity binding site, providing a statistical enhancement to K_D. This prediction is borne out by a more favorable entropy of binding relative to the other dimannosides (**12**–**14**).

Table 1: Thermodynamics of Binding for Lectins Isolated from *Dioclea grandiflora*, *Canavalia ensiformis* (Concanavalin A), and *Galanthus nivalis* (Snowdrop) with Various Oligosaccharides (kcal mol^{-1} at 25 °C)

	Dioclea			con A			snowdrop		
carbohydrate	ΔG	ΔH	$T\Delta S$	ΔG	ΔH	$T\Delta S$	ΔG	ΔH	$T\Delta S$
1	-4.8 ± 0.05	-7.8 ± 0.6	-2.9 ± 0.7	-5.3 ± 0.02	-6.8 ± 0.1	-1.5 ± 0.8	BD[a]		
2	NB[b]			NB[b]			NB[b]		
3	-4.2 ± 0.05	-4.4 ± 0.2	-0.1 ± 0.2	-4.6 ± 0.3	-5.3 ± 0.1	-0.7 ± 0.07	NB[b]		
4	NB[b]			NB[b]			NB[b]		
5	NB[b]			NB[b]			NB[b]		
6	NB[b]			NB[b]			NB[b]		
7	NB[b]			NB[b]			NB[b]		
8	NB[b]			NB[b]			NB[b]		
9	NB[b]			NB[b]			NB[b]		
10	-5.2 ± 0.03	-6.9 ± 0.2	-1.7 ± 0.2	-5.2 ± 0.02	-5.9 ± 0.1	-0.7 ± 0.08	NB[b]		
11	-5.9 ± 0.02	-6.4 ± 0.07	-0.5 ± 0.05	-6.8 ± 0.02	-7.0 ± 0.03	-0.2 ± 0.01	NB[b]		
12	-5.5 ± 0.03	-11.4 ± 0.03	-5.1 ± 0.8	-6.0 ± 0.02	-7.4 ± 0.08	-1.4 ± 0.06	-4.8 ± 0.07	-3.1 ± 0.2	$+1.7 \pm 0.1$
13	-5.0 ± 0.05	-8.4 ± 0.5	-3.5 ± 0.4	-5.0 ± 0.03	-7.4 ± 0.2	-2.3 ± 0.3	NB[b]		
14	-5.1 ± 0.03	-8.6 ± 0.3	-3.6 ± 0.2	-5.3 ± 0.04	-6.9 ± 0.4	-1.6 ± 0.4	-4.2 ± 0.2	-2.1 ± 1.0	$+2.1 \pm 0.8$
15	-4.3 ± 0.02	-4.4 ± 0.09	-0.1 ± 0.07	-4.4 ± 0.07	-3.7 ± 0.7	$+0.7 \pm 0.6$	NB[b]		
16	-4.2 ± 0.08	-4.4 ± 2.0	-0.2 ± 2.0	-4.4 ± 0.04	-4.6 ± 0.2	-0.2 ± 0.16	NB[b]		
17	-8.2 ± 0.05	-13.0 ± 0.08	-4.8 ± 0.03	-7.4 ± 0.07	-10.2 ± 0.1	-2.8 ± 0.03	-4.8 ± 0.06	-3.9 ± 0.4	$+0.9 \pm 0.34$

[a] BD indicates measurement is below the detection limits of the instruments. [b] NB indicates no binding.

Table 2: Binding Constants Derived from the Titration of the Lectins Isolated from *Dioclea grandiflora*, *Canavalia ensiformis* (Concanavalin A), and *Galanthus nivalis* (Snowdrop) with Various Oligosaccharides at 25 °C

	Dioclea			con A			snowdrop		
carbohydrate	[carb] (mM)	[lectin] (mM)	K (M^{-1})	[carb] (mM)	[lectin] (mM)	K (M^{-1})	[carb] (mM)	[lectin] (mM)	K (M^{-1})
1	20	0.33	3.6×10^3	26	0.37	7.6×10^3	50	1.06	BD[a]
2	18.4	0.30	NB[b]	18.4	0.33	NB[b]	18.4	0.67	NB[b]
3	16.1	0.26	1.2×10^3	16.1	0.28	2.4×10^3	16.1	1.07	NB[b]
4	17.4	0.23	NB[b]	17.4	0.58	NB[b]	17.4	0.34	NB[b]
5	15.1	0.26	NB[b]	15.1	0.34	NB[b]	15.1	0.34	NB[b]
6	16.1	0.26	NB[b]	16.1	0.43	NB[b]	16.1	0.34	NB[b]
7	20	0.30	NB[b]	20	0.43	NB[b]	20	0.34	NB[b]
8	20	0.26	NB[b]	20	0.43	NB[b]	20	0.34	NB[b]
9	20	0.31	NB[b]	20	0.32	NB[b]	20	0.67	NB[b]
10	20	0.30	6.3×10^3	20	0.43	6.3×10^3	20	0.34	NB[b]
11	19.8	0.27	2.0×10^4	19.8	0.34	1.0×10^5	26.3	0.69	NB[b]
12	8.7	0.21	1.0×10^4	20.6	0.32	3.0×10^4	17.3	0.35	3.3×10^3
13	15.3	0.30	5.1×10^3	15.8	0.43	5.1×10^4	15.8	0.34	NB[b]
14	14.6	0.26	5.3×10^3	20	0.36	6.1×10^3	19.9	0.35	1.2×10^3
15	19.2	0.29	1.4×10^3	19.2	0.32	1.6×10^3	19.2	1.07	NB[b]
16	26.3	0.23	1.2×10^3	22.5	0.90	1.7×10^3	26.3	0.34	NB[b]
17	15.2	0.20	7.4×10^5	16.5	0.32	2.6×10^5	16.2	0.24	3.0×10^3

[a] BD indicates measurement is below the detection limits of the instrument. [b] NB indicates no binding.

Thermodynamics of Binding of Lectins with Overlapping Binding Specificities but No Sequential or Structural Homology. The snowdrop lectin is one of a series of mannose-binding lectins present in the bulbs of members of the family Amaryllidaceae. The carbohydrate binding specificity of this group is distinct from that of the legume lectins in that it is limited to nonreducing terminal α-D-mannosyl groups; internal mannosyl residues, D-glucose, and *N*-acetyl-D-glucosamine do not interact with the snowdrop lectin (Shibuya *et al.*, 1988).

Kaku and Goldstein (1989, 1991) previously reported that the snowdrop lectin precipitates branched α-mannosides, but not α-glucosides. All β-glycosides are unreactive. The disaccharide methyl 3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside is the most effective disaccharide inhibitor, second overall only to the mannose trisaccharide.

Table 1 shows good agreement with Goldstein's data. The snowdrop lectin binds only α-anomers of high-mannose residues, while the β-anomer of methyl mannose and the reducing sugar *N*-acetylmannosamine are not bound. The lectin is solely mannose specific: α- and β-anomers of gluco- and galactopyranosides and the disaccharides maltose and isomaltose are not bound. The interaction between oligosaccharides and the snowdrop lectin is unusual in another respect: unlike the vast majority of known protein–carbohydrate interactions, snowdrop lectin/oligosaccharide binding interactions are characterized by an enthalpy of binding *less* negative than the free energy, with a *favorable* entropic contribution (Williams *et al.*, 1992).

The snowdrop lectin binds all mannose oligosaccharides with lower affinities than do the legume lectins. Although the binding of methyl α-D-mannopyranoside to the snowdrop lectin is detectable by agglutination assay, the association constant for the mannose monosaccharide falls below the sensitivity range of calorimetry. The value *C*, the product of the binding constant and the concentration of binding sites, should fall within the range of 1−1000 for any accurate deconvolution of calorimetric binding data, although thermicities are readily visible at *C* values considerably less than 1. For the snowdrop lectin, measurements were made at protein concentrations of greater than 1 mM. Because no binding was detectable at this protein concentration, we

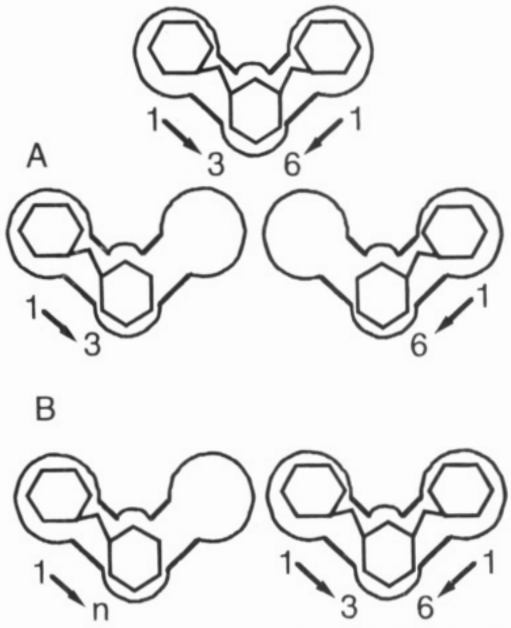

FIGURE 7: Schematic representation of lectin binding sites that share an affinity for the mannose trisaccharide methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside. (A) The carbohydrate binding site of the lectin from *Galanthus nivalis* has specific sites for the 1→3 and 1→6 arms of the trisaccharide, while the legume lectin binding sites (B) have a high-affinity monosaccharide binding site that binds the nonreducing terminus of all mannose disaccharides and an extended binding site that recognizes both nonreducing termini of the trisaccharide.

estimate the binding constant of methyl α-D-mannopyranoside to snowdrop lectin as less than 100 M^{-1}. Binding of the 1→2 and 1→4 disaccharides is not detectable by calorimetry, in sharp contrast to the behavior of the legume lectins. The trimannoside is bound with an efficacy approximately equal to that of the 1→3 disaccharide. These observations are consistent with Goldstein's report that the snowdrop lectin binds the trisaccharide 42 times more strongly than the monosaccharide methyl α-D-mannopyranoside, and that the 1→3 disaccharide binds 3 times more tightly than the 1→6 disaccharide (Shibuya *et al.*, 1988; Kaku & Goldstein, 1991).

Although the lectin shares a pronounced affinity for the mannose trisaccharide with the legume lectins, the thermodynamic pattern of binding interactions of the snowdrop lectin stands in stark contrast to that of the legume lectins. While concanavalin A and *Dioclea* have similar affinities for the mannose monosaccharide and all four dimannosides, interpretable in terms of a single high-affinity site, the snowdrop lectin displays an enhanced affinity for the disaccharides over the monosaccharide. Furthermore, *only* the 1→3 and 1→6 dimannosides are bound; clearly this pattern is inconsistent with a single high-affinity site. Rather these results are consistent with an extended binding site composed of distinct sites for the 1→6 and 1→3 arms (Figure 7). A binding site without this distinction would, in all probability, bind not only the 1→3 and 1→6 disaccharides but the 1→2 and 1→4 disaccharides as well. In addition, because the mannose trisaccharide could bind with either arm in a binding site, a statistical enhancement of the binding constant, like that seen for concanavalin A and the 1→2 disaccharide, would be apparent. The trimannoside has about the same binding constant as the 1→3 disaccharide; thus, the binding energies of the 1→3 and 1→6 disaccharides clearly are not simply additive. This observation is in accord with the concept of an "interaction energy" described by Jencks (1981).

FIGURE 8: Two views of concanavalin A showing the similarities between concanavalin A and the lectin from *Dioclea*. The Cα backbone from the Protein Data Bank file 2CTV is shown in pale gray at areas of difference (Hardman & Ainsworth, 1972; Ainouz *et al.*, 1987). The lighter sphere is Mn^{2+}, and the darker sphere is Ca^{2+}. Protein diagrams rendered by MolScript.

Thermodynamics of Binding of Lectins with Overlapping Binding Specificities and Sequential and Structural Homology. The more complex problem to be addressed in the preparation of highly specific carbohydrate ligands is that of highly homologous lectins with overlapping binding specificities, represented in our model by concanavalin A and the lectin from *Dioclea grandiflora*.

The lectin isolated from *Dioclea grandiflora* shares amino acid sequence, molecular mass, and subunit structure with concanavalin A (Moreira *et al.*, 1983). Differing by only 59 of 237 amino acids with no gaps, the structures are predicted to have essentially identical Cα traces. We also predict that the carbohydrate binding site remains in the same locus conserved across the legume lectins. The locations of the substitutions are superimposed on the concanavalin A structure in Figure 8. The only sequence difference close to the monosaccharide binding site is the exchange of Thr226 in concanavalin A for glycine in *Dioclea*. Thr226, despite its proximity to the monosaccharide binding site of con-

Table 3: Difference Calculations of Enthalpies and Free Energies of Binding for Concanavalin A and the Lectin Isolated from *Dioclea grandiflora* (kcal mol^{-1} at 25 °C)

carbohydrate[a]	$\Delta Hc - \Delta H_D$	$\Delta Gc - \Delta G_D$
αMeMan	+1.0	−0.5
1→6 diman	+1.8	−0.2
1→3 diman	+4.0	−0.6
triman	+4.5	+0.3

[a] αMeMan, methyl α-D-mannopyranoside; 1→6 diman, methyl 6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside; 1→3 diman, methyl 3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside; triman, methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside.

canavalin A, does not form a hydrogen bond with the C-2 hydroxyl of the monosaccharide methyl α-D-mannopyranoside (Derewenda *et al.*, 1989). The threonine hydroxyl is instead directed toward an adjacent subunit in the crystal cell. The orientation of Thr226 may be an artifact arising from crystal packing forces. However, a Monte Carlo simulation of carbohydrate binding indicates that the threonine hydroxyl adopts its outward orientation preferentially even in the absence of an adjacent subunit (B. D. Isbister and E. J. Toone, unpublished results).

The gross binding *patterns* observed for the two legume lectins are essentially identical: all mannose disaccharides bind with roughly the same affinity as methyl α-D-mannopyranoside, while the trimannoside is bound considerably more tightly. This binding pattern is consistent with an extended site composed of a high-affinity (monosaccharide) binding site and an extended low-affinity site that interacts with both nonreducing termini of the trisaccharide; only the nonreducing termini of the disaccharides are bound in the high-affinity site. The dimannoside binding constants are similar to that of methyl α-D-mannopyranoside. The *absolute values* of binding constants and binding enthalpies for complexation of mannose oligosaccharides to the lectin from *Dioclea* and concanavalin A differ markedly. The binding energy for the interaction of methyl α-D-mannopyranoside/*Dioclea* and all four disaccharides is reduced relative to that of concanavalin A by as much as 0.9 kcal mol^{-1}. The trimannoside, however, shows an enhanced affinity for *Dioclea* relative to concanavalin A of 0.8 kcal mol^{-1}. Furthermore, despite lower free energies of binding, the enthalpy of *Dioclea*/oligomannoside interactions is in all cases more negative than the analogous interaction with concanavalin A, by as much as 4 kcal mol^{-1} (Table 3). Thus, enthalpy−entropy compensation, long recognized as a function of *ligand* structure, is operative here as a function of *lectin* structure. Enthalpy−entropy compensation as a function of protein structure has previously been observed in mutagenesis studies (Kelley & O'Connell, 1993; Brummell *et al.*, 1993; Ito *et al.*, 1993).

The lectin from *Dioclea* also shares with concanavalin A the high affinity for the mannose trisaccharide, a trait that does not extend to other mannose-specific legume lectins, for example, the pea lectin (Rini *et al.*, 1993). Within the putative *Dioclea* lectin binding site, identified by homology with concanavalin A, there are three significant differences relative to concanavalin A: Ser205 and His206 in concanavalin A have been changed to His205 and Glu206, and Thr226 in concanavalin A has become Gly226 in *Dioclea* (Richardson *et al.*, 1984). Due to discrepancies found in reports of the *Dioclea* sequence, the change of His206 in concanavalin A to His205 in the lectin from *Dioclea* may not be a real one (Richardson *et al.*, 1984). Because the residues within the putative monosaccharide binding sites are identical, hydrogen-bonding *patterns* are presumably unchanged. Thus, changes in thermodynamic parameters can only reasonably be attributed to changes in the flexibility of the peptide loops that comprise the binding site, or to differences in solvation properties of the free or bound lectin: changes in one or both of these properties must account for the observed compensatory behavior.

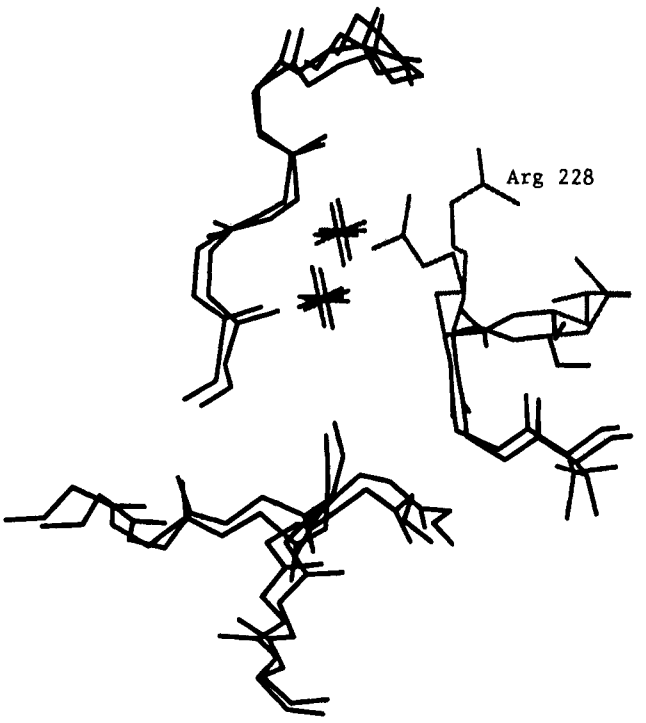

FIGURE 9: Overlay of unbound concanavalin A and concanavalin A bound with methyl α-D-mannopyranoside **1**. Overlay illustrates the 4.71-Å displacement of the Arg228 side chain upon binding the mannose monosaccharide.

Although there is significant preorganization of the concanavalin A monosaccharide binding site, several amino acid side chains move considerable distances to achieve optimal hydrogen bond geometries during complexation. The Arg228 side chain, for example, is displaced more than 5 Å during complexation to form hydrogen bonds with the C-3 hydroxyl of methyl α-D-mannopyranoside (Figure 9). Because of the altered ϕ/ψ space available to glycine relative to all other amino acids, the Thr to Gly modification at 226 should alter the accessible orientations of the Arg side chain. Thus, replacement of Thr226 by Gly could contribute to the differences in thermodynamics of association between concanavalin A and the lectin from *Dioclea*, despite the fact that the residue at 226 does not interact with the carbohydrate ligand. Because of increased loop flexibility, Arg228 is able to hydrogen bond to the monosaccharide more efficiently; the penalty for this enhanced hydrogen bonding is a more severe entropic loss as the Arg side chain is frozen.

Similarly, if the 1→3 arm of the trisaccharide is bound near His206 (in concanavalin A), as Carver asserts (Carver *et al.*, 1985), then the shift of the histidine from 206 in concanavalin A to 205 in the lectin from *Dioclea* could significantly improve hydrogen-bonding interactions with the disaccharide. This amino acid substitution, though, may not be a real one. Alternatively, the flexibilities of the two

proteins may differ significantly. Most of the sequence differences between the two lectins are in the turns connecting β-sheets; these changes could significantly affect the orientations of the binding loops, despite their distance from the binding site, both in sequence and in space. For example, the turn composed of residues 67−71 in concanavalin A (YPNAD) differs in both charge and available ϕ/ψ space from the analogous sequence in *Dioclea* (YSGSS). If the modifications change the flexibility of the loops composing the binding site, one predicted outcome would be an enhanced enthalpy of binding, from more efficient hydrogen bonding, accompanied by a compensating decrease in entropy, leading to a change in free energy less than the change in enthalpy.

An alternative source of the enthalpy−entropy compensation is a change in the behavior of the water of solvation near the two lectins during binding: solvation effects have previously been proposed as the origin of enthalpy−entropy compensation (Makhatadze & Privalov, 1993; Privalov & Makhatadze, 1993). *Accurate values of ΔC_p allow determination of the entropy change associated with the solvent release and the loss of configurational entropy, as well as the enthalpy of binding from solvent reorganization.* We demonstrate here how accurate calorimetric determination of ΔC_p allows molecular attribution of bulk thermodynamic properties and removes the ambiguity regarding the source of entropy−enthalpy compensation.

The total entropy of binding can be separated into terms for changes in solvation, and losses in configurational, rotational, and translational entropy, *i.e.*

$$\Delta S^\circ = \Delta S_{solv} + \Delta S_{config} + \Delta S_{rot} + \Delta S_{trans} \quad (1)$$

The sum of the configurational and solvent-related entropy terms has been referred to as the unitary contribution to ΔS°, and the sum of the rotational and translational entropy terms has been referred to as the cratic contribution (Kauzmann, 1959). The solvent contribution to ΔS° can be calculated from ΔC_p as

$$\Delta S_{solv} = \Delta S^*_{solv} + \Delta C_p \ln(T/T^*_s) \quad (2)$$

where T^*_s is the convergence temperature (Baldwin, 1986; Murphy *et al.*, 1990). This temperature, at which there is no solvent contribution to the hydrophobic entropy change, is 112.15 °C, or 385.15 K. ΔS^*_{solv} includes protonation and electrostatic contributions to entropy, and for the bindings discussed here is zero. The translational and rotational contribution to the overall entropy of binding is less well defined. Murphy and co-workers (Murphy *et al.*, 1993) have proposed that the translational entropy change is best approximated as the cratic contribution to the overall entropy of binding. For the case of a 1:1 complex with 1 mol/L as the standard state, the translational plus rotational entropy is then equal to −8 cal mol^{-1} K^{-1}. This value agrees well with the recent observation by Dunitz (Dunitz, 1994) that the loss of translational entropy for inclusion of water in crystal hydrates is between 0 and 7 eu per water. Spolar and Record (1994), however, suggest that the translational entropy may be as large as −50 eu, while Jencks has suggested a value near −35 eu (Jencks, 1975). For this discussion, the absolute value of $\Delta S_{trans+rot}$ is not important since $\Delta\Delta S$ is the term of interest, and ΔS_{trans} is presumably the same for both lectins. In our calculations here, we use the value −8 eu. Following calculation of ΔS_{solv} and assuming a value of $\Delta S_{trans+rot}$, the configurational contribution to the entropy of binding is available by subtraction. Calculated values for the entropic changes arising from solvation and configurational changes for binding of the lectin from *Dioclea* and concanavalin A with four mannose oligosaccharides are shown in Table 4.

Table 4: Calculated Entropic Contributions to the Binding of Mannose Oligosaccharides to Concanavalin A and the Lectin from *Dioclea grandiflora*

system[a]	ΔC_p[b]	ΔS°[b]	ΔS_{solv}[b]	ΔS_{config}[b]
con A				
αMeMan	−50	−4.9	+12.8	−9.7
1→6 diman	−44	−0.9	+11.3	−4.2
1→3 diman	−110	−4.8	+28.2	−25.0
triman	−93	−12.4	+28.2	−32.6
Dioclea				
αMeMan	−56	−9.8	+14.4	−16.2
1→6 diman	−22	−12.0	+5.6	−9.6
1→3 diman	−40	−17.2	+10.2	−19.4
triman	−96	−26.4	+24.6	−43.0

[a] Abbreviations same as Table 3. [b] cal mol^{-1} deg^{-1}.

Both configurational and solvation entropies scale roughly as the size of the ligand, presumably a reflection of both the number of solvent molecules stripped and the number of freely rotating bonds restricted during binding. Table 4 also shows that, with the exception of the 1→3 dimannoside, differences in *configurational* entropy are responsible for the observed enthalpy−entropy compensation. For the 1→3 dimannoside, the more unfavorable entropic term accompanying *Dioclea* lectin association relative to concanavalin A apparently results from a greatly reduced solvation contribution to the entropy of binding.

Changes in protein backbone flexibility certainly offer a significant, but not all-encompassing, explanation for the dramatic increase in both the enthalpy and the entropy of binding for the lectin from *Dioclea* relative to concanavalin A. On the basis of studies of the deoxy Le^b-OMe-GSIV binding interactions, Lemieux *et al.* (Spohr *et al.*, 1985) suggested small changes in "nonessential" hydroxyls, *i.e.*, those that extend out of the binding site, perturb the water associated with the binding site. Mutations to or in the vicinity of the binding site could have a similar effect. By using thermodynamic solvent isotope effects, we have recently shown that the enthalpy of binding arising from solvent reorganization is related to ΔC_p. The solvation contribution to the enthalpy of binding for each system is shown in Table 5. As we have previously observed, solvation effects account for roughly 25% of the net binding enthalpy in protein−carbohydrate interactions (Chervenak & Toone, 1994). Furthermore, the differences in solvation enthalpy throughout are small, probably not outside the errors associated with this method. Thus, although changes in solvent structure contribute significantly to the enthalpy of binding, they do not contribute strongly to enthalpy−entropy compensation. The enhanced enthalpy of binding of *Dioclea* relative to concanavalin A must therefore represent enthalpically more favorable solute−solute interactions. Similar conclusions, that enthalpy−entropy compensation arises from differential protein−ligand interactions offset by unfavorable

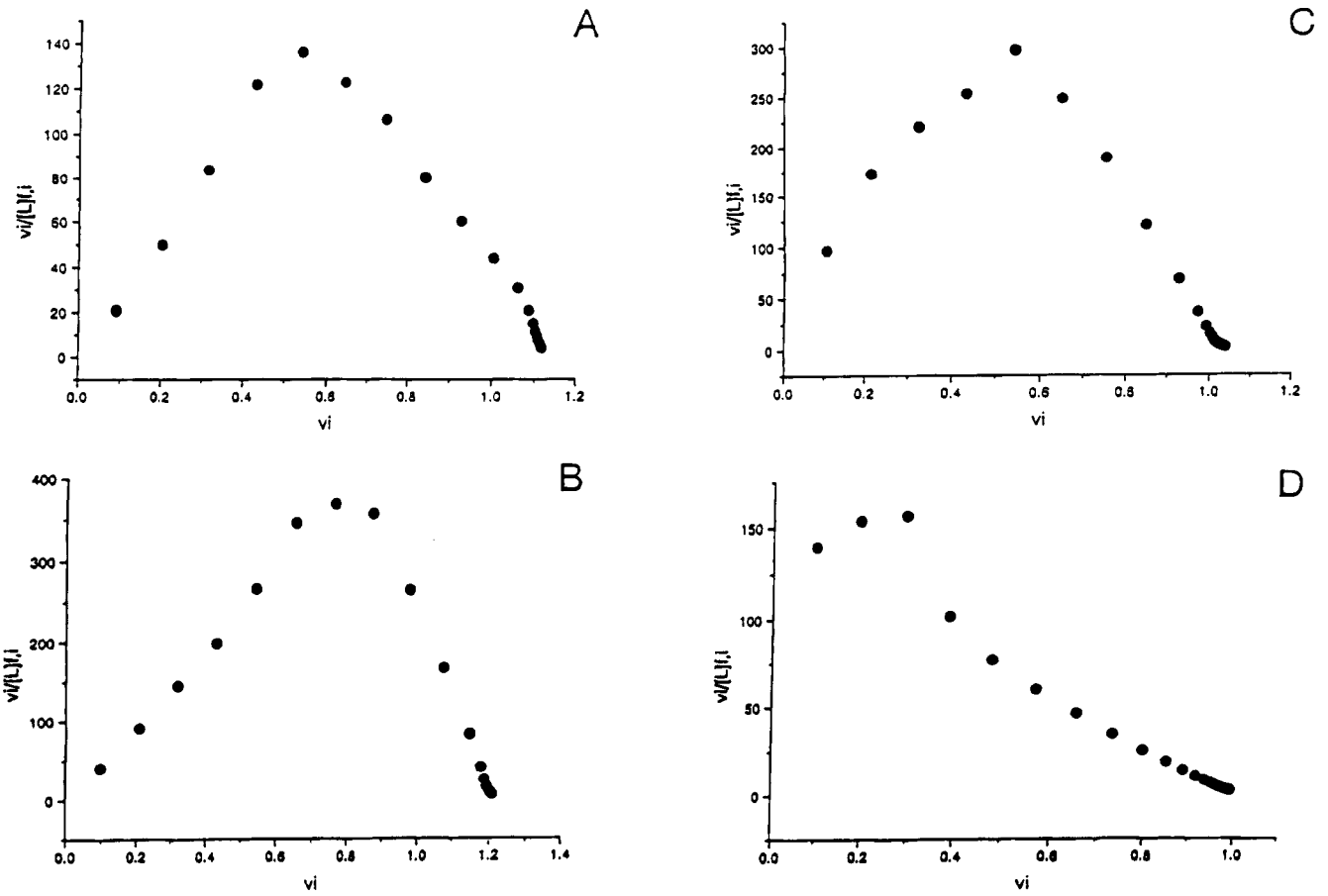

FIGURE 10: Scatchard plot for the binding of the lectin from *Dioclea* to methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside **17** at pH 6.92: (A) 8.1, (B) 14.8, (C) 25.2, and (D) 45.0 °C.

configurational entropy, were reached by other investigators (Kelley & O'Connell, 1993; Brummell *et al.*, 1993; Ito *et al.*, 1993).

Scatchard plots for the binding of the mannose trisaccharide to the lectin from *Dioclea* show significant downward curvature; this curvature is much stronger than that seen in concanavalin A/trimannoside plots. Greater positive cooperativity is consistent with greater reorganization of the binding site during saccharide binding by *Dioclea* than by concanavalin A, if protein relaxation dynamics are the source of cooperativity. To test this hypothesis, we generated Scatchard plots for *Dioclea*/trimannoside binding as a function of temperature. Figure 10 shows that the curvature of the plots decreases with increasing temperature. This observation supports the origin of the observed cooperativity as a dynamic phenomenon, which is in turn consistent with the thermodynamic parameters for oligosaccharide complexation that suggest differences in protein flexibility between concanavalin A and *Dioclea*.

CONCLUSIONS

Our examination of the thermodynamics of different molecular motifs that bind the same epitope provides evidence that nature has designed several unique ways of binding the same shape. Both the legume lectins concanavalin A and the lectin from *Dioclea grandiflora*, as well as the snowdrop lectin from *Galanthus nivalis*, recognize the trisaccharide methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside. The binding patterns of legume lectin are consistent with a binding site composed of a high-affinity site and a weaker extended binding site. Binding the same oligosaccharide to the snowdrop lectin suggests two separate sites specifically tailored for the 1→3 and 1→6 arms of the mannose trisaccharide.

Concanavalin A and the lectin from *Dioclea*, despite their shared sequence homology, bind the same oligosaccharides, and particularly the trisaccharide, very differently. Alterations in amino acid sequence may result in greater protein backbone flexibility that is manifested in a change in carbohydrate binding. Enthalpy−entropy compensation as a function of lectin structure is most likely the result of enhanced solute−solute interactions, offset by a more unfavorable configurational entropy of binding. In order to determine the specifics of these binding interactions, a complete thermodynamic profile that includes a knowledge of ΔC_p is required.

REFERENCES

Agrawal, B. B. L., & Goldstein, I. J. (1967) *Biochim. Biophys. Acta 147*, 262.

Ainouz, I. L., Moreira, R. A., Campos, F. D. A. P., Richardson, M., Begbie, R., Stewart, J. C., Watt, W. B., & Pusztai, A. (1987) *Phytochemistry 26*, 1435.

Ambrosino, R., Barone, G., Castronuovo, G., Ceccarini, C., Cultera, O., & Elia, V. (1987) *Biochemistry 26*, 3971.

Anderson, O., Laursen, S. B., Svehag, S.-E., Holmskov, U., & Theil, S. (1991) in *Lectin Reviews, Volume 1* (Kilpatrick, D. C., Van Driessche, E., & Bøg-Hansen, T. C., Eds.) p 41, Sigma, St. Louis.

Baldwin, R. L. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 8069.

Bertels, A., DeGreve, H., & Lintermans, P. (1991) in *Lectin Reviews, Volume 1* (Kilpatrick, D. C., Van Driessche, E., & Bøg-Hansen, T. C., Eds.) p 53, Sigma, St. Louis.

Brewer, C. F., Bhattacharyya, L., Brown, R. D., & Koenig, S. H. (1985) *Biochem. Biophys. Res. Commun. 127*, 1066.
Brummell, D. A., Sharma, V. P., Anand, N. N., Bilous, D., Dubuc, G., Michniewicz, J., MacKenzie, C. R., Sadowska, J., Sigurskjold, B. W., Sinnott, B., Young, N. M., Bundle, D. R., & Narang, S. A. (1993) *Biochemistry 32*, 1180.
Carver, J. P., Mackenzie, A. E., & Hardman, K. D. (1985) *Biopolymers 24*, 49.
Chervenak, M. C., & Toone, E. J. (1994) *J. Am. Chem. Soc. 116*, 10533.
Citri, N. (1973) *Adv. Enzymol. 29*, 397.
Cunningham, B. A., Wang, J. L., Pflumm, M. N., & Edelman, G. M. (1972) *Biochemistry 11*, 3233.
Delbaere, L. T. J., Vandonselaar, M., Prasad, L., Quail, J. W., Wilson, K. S., & Dauter, Z. (1993) *J. Mol. Biol. 230*, 950.
Derewenda, Z., Yariv, J., Helliwell, J. R., Kalb, A. J., Dodson, E. J., Papiz, M. Z., & Campbell, J. (1989) *EMBO J. 8*, 2189.
Drickhamer, K., & Taylor, M. E. (1993) *Annu. Rev. Cell Biol. 9*, 237.
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956) *Anal. Chem. 28*, 350.
Dunitz, J. D. (1994) *Science 264*, 670.
Edelhoch, H. (1967) *Biochemistry 6*, 1948.
Eftink, P. L., & Biltonen, R. (1980) in *Biological Microcalorimetry* (Beezer, A. E., Ed.) p 343, Academic, London.
Feizi, T., & Childs, R. A. (1987) *Biochem J. 245*, 1.
Gabius, H.-J. & Gabius, S. (1991) in *Lectin Reviews, Volume 1* (Kilpatrick, D. C., Van Driessche, E., & Bøg-Hansen, T. C., Eds.) p 91, Sigma, St. Louis.
Grant, C. W. M., & Peters, M. W. (1984) *Biochim. Biophys. Acta 779*, 403.
Hardman, K. D., & Ainswoth, C. F. (1972) *Biochemistry 11*, 4910.
Hiraizumi, S., Takasaki, S., Ohuchi, N., Harada, Y., Nose, M., Mori, S., & Kobata, A. (1992) *J. Cancer Res. 83*, 1063.
Ingram, G. A., & Molyneux, D. H. (1991), in *Lectin Reviews, Volume 1* (Kilpatrick, D. C., Van Driessche, E., & Bøg-Hansen, T. C., Eds.) p 103, Sigma, St. Louis.
Itzkowitz, S. H., Bloom, E. J., Kokal, W. A., Modin, G., Hakomori, S., & Kim, Y. S. (1990) *Cancer 66*, 1960.
Jencks, W. P. (1975) in *Advances in Enzymology, Volume 43* (Meister, A., Ed.) p 219, Wiley, New York.
Jencks, W. P. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4046.
Kaku, H., & Goldstein, I. J. (1989) *Methods Enzymol. 179*, 327.
Kaku, H., & Goldstein, I. J. (1991) *Carbohydr. Res. 213*, 109.
Karlsson, K.-A. (1989) *Annu. Rev. Biochem. 58*, 309.
Kauzmann, W. (1959) *Adv. Protein Chem. 14*, 1.
Kelley, R. F., & O'Connell, M. P. (1993) *Biochemistry 32*, 6828.
Ito, W., Iba, Y., & Kurosawa, Y. (1993) *J. Biol. Chem. 268*, 16639.
Liener, I. E. (1976) in *Concanavalin A as a Tool* (Bittinger, H., & Schnebli, H. P., Eds.) p 17, Wiley, New York.
Liener, I. E., Sharon, N., & Goldstein, I. J. (1986) *The Lectins: Properties, Functions, and Applications in Biology and Medicine*, Academic, New York.
Lis, H., & Sharon, N. (1986) *Annu. Rev. Biochem. 55*, 35.
Mackenzie, A. E. (1986), Ph.D. Dissertation, University of Toronto.
Makhatadze, G. I., & Privalov, P. L. (1993) *J. Mol. Biol. 232*, 639.
Mandal, D. K., Kishore, N., & Brewer, C. F. (1994) *Biochemistry 33*, 1149.
Manocha, M. S., & Chen, Y. (1990) *Can. J. Microbiol. 36*, 69.
Moreira, R. A., Barros, A. C. H., Stewart, J. C., & Pusztai, A. (1983) *Planta 158*, 63.
Muramatsu, T. (1993) *Glycobiology 3*, 298.
Murphy, K. P., Privalov, P. L., & Gill, S. J. (1990) *Science 247*, 559.
Murphy, K. P., Xie, D., Thompson, K. S., Amzel, L. M., & Freire, E. (1993) *Proteins: Struct., Funct., Genet. 18*, 63.
Privalov, P. L., & Makhatadze, G. I. (1993) *J. Mol. Biol. 232*, 660.
Richardson, M., Campos, F. D. A. P., Moreira, R. A., Ainouz, I. L., Begbie, R., Watt, W. B., & Pusztai, A. (1984) *Eur. J. Biochem. 144*, 101.
Rini, J. M., Hardman, K. D., Einspahr, H., Suddath, F. L., & Carver, J. P. (1993) *J. Biol. Chem. 268*, 10126.
Saha, S. K., & Brewer, C. F. (1994) *Carbohydr. Res. 254*, 157.
Schwarz, F. P., Puri, K. D., Bhat, R. G., & Surolia, A. J. (1993) *J. Biol. Chem. 268*, 7668.
Sharon, N., & Lis, H. (1989) *Lectins*, Chapman and Hall, New York.
Shibuya, N., Goldstein, I. J., Van Damme, E. J. M., & Peumans, W. J. (1988) *J. Biol. Chem. 263*, 728.
Spiess, M. (1990) *Biochemistry 29*, 10009.
Spohr, U., Hindsgaul, O., & Lemieux, R. U. (1985) *Can. J. Chem. 53*, 2644.
Spolar, R. S., & Record, M. T. (1994) *Science 263*, 777.
Sumner, J. B., & Howell, S. F. (1936) *J. Bacteriol. 32*, 262.
Takeichi, M. (1991) *Science 251*, 1451.
Van Damme, E. J. M., Allen, A. K., & Peumans, W. J. (1987) *FEBS Lett. 215*, 140.
Varki, A. (1993) *Glycobiology 3*, 97.
Weber, G. (1975) *Adv. Prot. Chem. 29*, 1.
Williams, B. A., Chervenak, M. C., & Toone, E. J. (1992) *J. Biol. Chem. 267*, 22907.
Wiseman, T., Williston, S., Brandts, J. F., & Lin. L.-N. (1989) *Anal. Biochem. 179*, 131.

BI942401V