The Man/Glc-specific seed lectin from *Dioclea grandiflora* (DGL) is a member of the Dioecieinae subtribe that includes the jack bean lectin concanavalin A (ConA). Both DGL and ConA bind with high affinity to the “core” trimannoside moiety, 3,6-di-O-(α-D-mannopyranosyl)-α-D-mannopyranoside, which is present in asparaginereleased carbohydrates. Recent hemagglutination inhibition studies suggest that DGL and ConA recognize similar epitopes of the trisaccharide but possess different binding specificities for complex carbohydrates (Gupta, D., Oscarson, S., Raju, T. S., Stanley, P., Toone, E. J., and Brewer, C. F. (1996) *Eur. J. Biochem.* 242, 320–326). In the present study, we have used isothermal titration microcalorimetry to determine the thermodynamics of binding of DGL to a complete set of monodeoxy analogs of the core trimannoside as well as a tetra- deoxy analog. The thermodynamic data indicate that DGL recognizes the 2-, 3-, 4-, and 6-hydroxy groups of the α(1,6) Man residue, the 3- and 4-hydroxy group of the α(1,3) Man residue, and the 2- and 4-hydroxyl groups of the central Man residue of the trimannoside. The thermodynamic data for the tetra- deoxy analog lacking the 3- and 4-hydroxy group of the α(1,3) Man residue, and the 2- and 4-hydroxy groups of the central Man residue of the trimannoside are consistent with the involvement of these hydroxyl groups in binding. While the overall pattern of data for DGL binding to the deoxy analogs is similar to that for ConA (Gupta, D., Dam, T. K., Oscarson, S., and Brewer, C. F. (1997) *J. Biol. Chem.* 272, 6388–6392), differences exist in the data for certain monodeoxy analogs binding to the two lectins. Differences are also observed in the thermodynamics of binding of DGL and ConA to a biantennary complex carbohydrate. In the following paper (Rozwarski, D. A., Swami, B. M., Brewer, C. F., and Sacchettini, J. C. (1998) *J. Biol. Chem.* 273, 32818–32825), the x-ray crystal structure of DGL complexed to the core trimannoside is presented, and a comparison is made of the thermodynamic binding data for DGL and ConA as well as the structures of their respective trimannoside complexes.

The seed lectin from *Dioclea grandiflora* (DGL) is a Man/Glc-binding protein obtained from northeast Brazil and is a member of a large group of lectins from the subtribe Dioecieinae. DGL is devoid of covalently linked carbohydrate and is reported to be a tetramer with a molecular mass of 100,000 Da (1). As with other legume lectins, DGL requires Ca²⁺ and a transition metal ion for its binding activity. The lectin possesses a high degree of sequence homology with the jack bean lectin, concanavalin A (ConA), another member of the Dioecieinae subtribe, differing in 52 out of 237 residues (2). Six of the seven residues that have been implicated as ligands for the Ca²⁺ and transition metal ion sites are conserved, and the seven amino acid residues surrounding the carbohydrate binding site of ConA are also conserved in DGL (2).

Despite similarities in the relatively conserved structures of the two lectins and their similar Man/Glc binding specificities, DGL and ConA possess differences in their binding specificities for larger N-linked carbohydrates including complex carbohydrate 16 (Fig. 1) (3). Furthermore, both ConA and DGL possess different biological activities such as histamine release from rat peritoneal mast cells (4). Thus, it is important to investigate the detailed carbohydrate binding specificity of DGL in order to understand the molecular basis for its biological activities relative to other members of the Dioecieinae subtribe including ConA.

DGL and ConA, like other members of the Dioecieinae subtribe (5), bind with high affinity to the trimannoside moiety, 3,6-di-O-(α-D-mannopyranosyl)-α-D-mannopyranosane, which is present in the core region of N-linked carbohydrates (6, 7). In order to gain insight into the detailed binding specificities of DGL and ConA, a series of mono and deoxy derivatives (2–14) of methyl 3,6-di-O-(α-mannopyranosyl)-α-mannopyranosane (Fig. 1) were synthesized and tested for their ability to bind to both lectins as determined by hemagglutination inhibition methods (3, 5). The results suggest that both ConA and DGL have similar extended binding sites that recognize the same epitopes of the trimannoside.

In the present study, isothermal titration microcalorimetry (ITC) is used to determine the thermodynamics of DGL binding to monodeoxy analogs 2–11 and tetra- deoxy analog 15, as well as biantennary complex carbohydrate 16 (Fig. 1). The thermodynamic data for DGL is compared with corresponding data recently reported for ConA as well as data for 15 reported in the present study (8). In the following paper (26), the x-ray crystal structure of the DGL complex with trimannoside 1 is pre-
sent, and a comparison is made of the solution thermodynamic data and structure of the DGL complex. Differences in the thermodynamic data for DGL and ConA binding to the deoxy analogs as well as to 16 are compared with the structures of the two lectins complexed to the trimannoside.

EXPERIMENTAL PROCEDURES

DGL was isolated from D. grandiflora seeds obtained from north-eastern Brazil (Albano Ferreira Martin Ltd., Sao Paulo, Brazil) as described previously (1). The concentration of DGL was determined spectrophotometrically at 280 nm using $A_{1%}^{1%} = 12.0$ at pH 7.2 and expressed in terms of monomer ($M = 25,000$) (1). ConA was prepared from jack bean (Canavalia ensiformis) seeds (Sigma) according to the method of Agrawal and Goldstein (9). The concentration of ConA was determined spectrophotometrically at 280 nm using $A_{1%}^{1%} = 13.7$ at pH 7.2 (10) and expressed in terms of monomer ($M = 25,800$).

RESULTS AND DISCUSSION

DGL Binding to Mono- and Oligosaccharides—In order to compare thermodynamic data for DGL binding to simple sugars with larger N-linked carbohydrates including trimannoside 1 and its deoxy analogs, ITC data for the mono- and disaccharides were obtained from OMEGA Microlorimeter from Microcal, Inc. (Northampton, MA). In individual titrations, injections of 4 μl of carbohydrate were added from the computer-controlled 250-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. An example of an ITC experiment is shown in Fig. 2 for deoxy analog 4 with DGL at 27 °C. 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 $\Delta H$ (enthalpy change in kcal mol$^{-1}$), $K_a$ (association constant in M$^{-1}$), and $n$ (number of binding sites/monomer) as adjustable parameters. The quantity $c = K_a M_i(0)$, where $M_i(0)$ is the initial macromolecule concentration, is of importance in titration microcalorimetry (14). All experiments were performed with $c$ values $< 0.1$ mol,$^{-1}$ K$^{-1}$.

Fig. 2. Calorimetric titration profile of DGL with α(1→3)4-deoxy analog 4 at 27 °C. Top panel, data obtained for 30 automatic injections, each 4 μl of 4; bottom panel, the integrated curve showing experimental points (■) and the best fit (line). The buffer was 0.1 M Hepes containing 0.9 M NaCl, 1 mM MnCl$_2$, and 1 mM CaCl$_2$ at pH 7.2.

$\Delta G = \Delta H - T\Delta S = -RT\ln K_a$ (Eq. 1) where $\Delta G$, $\Delta H$, and $\Delta S$ are the changes in free energy, enthalpy, and entropy of binding. $T$ is the absolute temperature, and $R = 1.98$ cal K$^{-1}$ mol$^{-1}$.

1c m

binding specificity of D. grandiflora lectin 32813
TABLE I

Thermodynamic parameters derived from the titration of DGL at pH 7.2 with saccharides at 27 °C

The buffer was 0.1 M Hepes containing 0.9 M NaCl, 1 mM Mn²⁺, and 1 mM Ca²⁺ at pH 7.2. Values of n were between 0.99 and 1.02 in all cases.

| Carbohydrate (abbreviated form) | Carbohydrate concentration | Lectin concentration | $K_a$ |
|---------------------------------|---------------------------|----------------------|-------|
|                                 | nm                        | mM                   | $m^{-1} \times 10^{-4}$ |
| MecMan                          | 49.0                      | 0.41                 | 0.46  |
| MecGlc                          | 60.0                      | 0.49                 | 0.28  |
| Me2deGlc                        | 50.0                      | 0.29                 | 0.22  |
| Manα(1–2)ManαOMe                | 16.0                      | 0.36                 | 3.0   |
| Manα(1–3)ManαOMe                | 16.0                      | 0.29                 | 1.8   |
| Manα(1–6)ManαOMe                | 17.0                      | 0.29                 | 0.56  |
| 1, trimannoside                 | 7.0                       | 0.13                 | 122   |
| 2, α(1–3)2-deoxy                | 5.7                       | 0.19                 | 79    |
| 3, α(1–3)3-deoxy                | 10.5                      | 0.21                 | 5.4   |
| 4, α(1–3)4-deoxy                | 6.2                       | 0.23                 | 56    |
| 5, α(1–3)6-deoxy                | 6.7                       | 0.21                 | 74    |
| 6, α(1–6)2-deoxy                | 9.3                       | 0.24                 | 21.3  |
| 7, α(1–6)3-deoxy                | 15.9                      | 0.27                 | 2.57  |
| 8, α(1–6)4-deoxy                | 9.7                       | 0.18                 | 2.4   |
| 9, α(1–6)6-deoxy                | 9.8                       | 0.22                 | 1.89  |
| 10, "core"2-deoxy              | 14.7                      | 0.20                 | 24.9  |
| 11, "core"4-deoxy              | 17.6                      | 0.31                 | 10.8  |
| 15, α(1–3)3,4-deoxy,"core"2,4-deoxy | 22.0                    | 0.46                 | 1.54  |
| 16, complex carbohydrate       | 13.8                      | 0.51                 | 4.7   |

| Carbohydrate (abbreviated form) | $-\Delta G$ | $\Delta G^\circ$ | $-\Delta H^\circ$ | $\Delta H^\circ$ | $-T\Delta S^\circ$ |
|---------------------------------|-------------|------------------|-------------------|-----------------|------------------|
| MecMan                          | 4.9         | 8.2              | 3.3               |                 |
| MecGlc                          | 4.7         | 5.0              | 0.3               |                 |
| Me2deGlc                        | 4.6         | 7.5              | 2.9               |                 |
| Manα(1–2)ManαOMe                | 6.1         | 9.9              | 3.8               |                 |
| Manα(1–3)ManαOMe                | 5.8         | 10.1             | 4.3               |                 |
| Manα(1–4)ManαOMe                | 5.1         | 8.4              | 3.3               |                 |
| 1, trimannoside                 | 8.3         | 16.2             | 7.9               |                 |
| 2, α(1–3)2-deoxy                | 8.0         | 15.2             | 1.0               | 7.2             |
| 3, α(1–3)3-deoxy                | 6.5         | 11.0             | 5.2               | 4.5             |
| 4, α(1–3)4-deoxy                | 7.8         | 14.6             | 6.8               |                 |
| 5, α(1–3)6-deoxy                | 8.0         | 15.1             | 1.1               | 7.1             |
| 6, α(1–6)2-deoxy                | 7.3         | 12.8             | 3.4               | 5.5             |
| 7, α(1–6)3-deoxy                | 6.0         | 10.0             | 6.2               | 4.0             |
| 8, α(1–6)4-deoxy                | 5.9         | 10.4             | 5.8               | 4.5             |
| 9, α(1–6)6-deoxy                | 5.8         | 9.5              | 6.4               | 4.0             |
| 10, "core"2-deoxy              | 7.3         | 14.8             | 1.4               | 7.5             |
| 11, "core"4-deoxy              | 6.8         | 12.8             | 3.4               | 6.0             |
| 15, α(1–3)3,4-deoxy,"core"2,4-deoxy | 5.7         | 9.9              | 6.3               | 4.2             |
| 16, complex carbohydrate       | 6.4         | 11.6             | 11.6              | -1.8            |

Errors in $K_a$ values were between 2 and 10%.

Errors in $\Delta H$ and $T\Delta S$ were ±0.1–0.2 kcal mol⁻¹.

Relative to 1.

rides in Table I were obtained. $K_a$ and $\Delta H$ values of 4.6 × 10³ m⁻¹ and −8.2 kcal mol⁻¹, respectively, for DGL binding to MecMan agree well with published values by Chervenak and Toone (6). For comparison, values of $K_a$ and $\Delta H$ for ConA binding to MecMan, which are very similar to that for DGL, are shown in Table II. $K_a$ and $\Delta H$ values for MecGlc in Table I also agree with values reported by Chervenak and Toone (6) and are somewhat lower than those for MecMan. Me2deGlc also shows somewhat reduced $K_a$ (2.2 × 10³ m⁻¹) and $\Delta H$ (−7.5 kcal mol⁻¹) values relative to that for MecMan, similar to ConA (15). Data for α(1–2), α(1–3), and α(1–6) mannosides in Table I indicate slight enhanced affinities of DGL for the α(1–2) and α(1–3) disaccharides, relative to MecMan. The slightly greater $\Delta H$ values for Manα(1–2)ManαOMe (−9.9 kcal mol⁻¹) and Manα(1–3)ManαOMe (−10.6 kcal mol⁻¹) relative to MecMan (−8.2 kcal mol⁻¹) suggest some extended site binding of the two disaccharides to DGL. Values of $K_a$ and $\Delta H$ in Table I for the α(1–2) disaccharide are greater than those previously reported (6), while those for the α(1–3) and α(1–6) mannosides are similar to those reported by these workers.

**DGL Binding to Trimmannoside I**—ITC data in Table I show that DGL binds to I with a change in enthalpy ($\Delta H$) of −16.2 kcal mol⁻¹ and a $K_a$ of 1.2 × 10⁶ m⁻¹. $\Delta H$ for I is −8.0 kcal mol⁻¹ greater than that of MecMan (Table I), and the affinity constant of DGL for I is 270-fold greater than that of the monosaccharide. These results generally agree with the $\Delta H$ and $K_a$ values for I reported by Chervenak and Toone (6). The thermodynamic data in Table I indicate that the high affinity of DGL for I, relative to MecMan, is due to extended site interactions. These results can be compared with ITC data for ConA (Table II, for comparison), which shows a $\Delta H$ for I that is −6.5 kcal mol⁻¹ greater than that of MecMan and a 60-fold greater $K_a$ for I compared with the monosaccharide (8, 15). Thus, DGL shows higher affinity and a greater −$\Delta H$ value for I than ConA.

**Binding of Monodeoxy Analogs of Trimmannoside I**—Hemagglutination inhibition studies (3) have recently shown that DGL and ConA have similar patterns of binding toward deoxy trimannoside analogs 2–13 in Fig. 1. In order to further characterize the binding epitopes of I with DGL, ITC has been used to determine the thermodynamics of binding of monodeoxy analogs 2–11 and tetra deoxy analog 15 with the lectin.

Interactions of the α(1–3) arm of trimannoside I with DGL were determined using the α(1–3)2-deoxy (2), α(1–3)3-deoxy...
TABLE II

Thermodynamic parameters derived from the titration of ConA at pH 7.2 with saccharides at 27 °C

The buffer was 0.1 M Hepes containing 0.9 M NaCl, 1 mM Mn²⁺, and 1 mM Ca²⁺ at pH 7.2. Values of n were between 0.99 and 1.02 in all cases.

| Carbohydrate (abbreviated form) | Carbohydrate concentration | Lectin concentration | \( K_a \) |
|---------------------------------|----------------------------|---------------------|---------|
|                                 | mm                        | mm                  | \( \mu M \times 10^{-4} \) |
| MecMan                          | 46.0                      | 0.48                | 1.2     |
| 1, trimannoside                 | 7.2                       | 0.21                | 39.0    |
| 2, α(1→3)-deoxy                 | 5.2                       | 0.20                | 19.0    |
| 3, α(1→3)-deoxy                 | 9.0                       | 0.14                | 4.8     |
| 4, α(1→3)-deoxy                 | 9.0                       | 0.24                | 9.2     |
| 5, α(1→3)-deoxy                 | 6.4                       | 0.15                | 39.6    |
| 6, α(1→3)-deoxy                 | 6.0                       | 0.20                | 16.0    |
| 7, α(1→6)-deoxy                 | 14.5                      | 0.33                | 3.9     |
| 8, α(1→6)-deoxy                 | 7.0                       | 0.23                | 2.54    |
| 9, α(1→6)-deoxy                 | 12.5                      | 0.33                | 3.01    |
| 10, α(1→3)-deoxy                 | 12.5                      | 0.24                | 11.7    |
| 11, α(1→3)-deoxy                 | 9.3                       | 0.26                | 4.63    |
| 12, α(1→3)-deoxy,α(1→4)-deoxy   | 42.5                      | 0.54                | 3.60    |
| 13, α(1→3)-deoxy,α(1→4)-deoxy   | 7.0                       | 0.24                | 1.93    |
| 14, α(1→3)-deoxy,α(1→4)-deoxy   | 45.0                      | 0.75                | 0.99    |
| 15, α(1→3)-deoxy,α(1→4)-deoxy   | 14.5                      | 0.62                | 1.30    |
| 16, Complex carbohydrate         | 8.0                       | 0.42                | 14.0    |

| Carbohydrate (abbreviated form) | Δ\( G \) | ΔΔ\( G \) | −Δ\( H \) | ΔΔ\( H \) | −TΔS |
|---------------------------------|---------|---------|----------|---------|-------|
| MecMan                          | 5.6     | 8.4     | 14.7     | 7.1     |
| 1, trimannoside                 | 7.6     | 14.7    | 0.5      | 0.1     |
| 2, α(1→3)-deoxy                 | 7.2     | 0.4     | 14.1     | 0.6     | 6.9   |
| 3, α(1→3)-deoxy                 | 6.4     | 1.4     | 11.0     | 3.2     | 4.6   |
| 4, α(1→3)-deoxy                 | 6.8     | 1.0     | 12.3     | 2.2     | 5.5   |
| 5, α(1→3)-deoxy                 | 7.7     | −0.1    | 14.0     | 0.7     | 6.3   |
| 6, α(1→3)-deoxy                 | 7.1     | 0.5     | 14.0     | 0.7     | 6.2   |
| 7, α(1→3)-deoxy                 | 6.3     | 1.3     | 11.2     | 3.5     | 4.9   |
| 8, α(1→6)-deoxy                 | 6.0     | 1.6     | 11.7     | 3.0     | 5.7   |
| 9, α(1→6)-deoxy                 | 6.1     | 1.5     | 11.6     | 3.1     | 5.5   |
| 10, α(1→3)-deoxy                 | 6.9     | 0.7     | 13.4     | 1.3     | 6.5   |
| 11, α(1→3)-deoxy                 | 6.4     | 1.2     | 12.1     | 2.6     | 5.7   |
| 12, α(1→3)-deoxy,α(1→4)-deoxy   | 6.2     | 1.4     | 10.6     | 4.1     | 4.4   |
| 13, α(1→3)-deoxy,α(1→4)-deoxy   | 5.9     | 1.7     | 9.7      | 5.0     | 3.8   |
| 14, α(1→3)-deoxy,α(1→4)-deoxy   | 5.5     | 2.1     | 8.7      | 6.0     | 3.2   |
| 15, α(1→3)-deoxy,α(1→4)-deoxy   | 5.6     | 2.0     | 8.9      | 5.8     | 3.3   |
| 16, complex carbohydrate         | 8.4     | −0.8    | 10.6     | 4.1     | 2.2   |

\( a \) Errors in \( K_a \) values were between 2 and 10%.

\( b \) Present study.

\( c \) Data taken from Mandal et al. (25) and included here for comparison.

\( d \) Data taken from Gupta et al. (8) and included here for comparison.

\( e \) Errors in \( ΔH \) and \( TΔS \) were ±0.1 to ±0.2 kcal mol⁻¹.

\( f \) Relative to 1.

(3), α(1→3)-deoxy (4), and α(1→3)-deoxy (5) derivatives (Fig. 1). Table I shows that the \( K_a \) values of 2 (7.9 × 10⁻³ M⁻¹) and 5 (7.4 × 10⁻³ M⁻¹) are slightly lower than that of 1 (1.2 × 10⁻² M⁻¹) and that the Δ\( H \) values for 2 (−15.2 kcal mol⁻¹) and 5 (−15.1 kcal mol⁻¹) are also only slightly reduced from that of 1 (−15.7 kcal mol⁻¹). On the other hand, 3 shows a 20-fold decrease in \( K_a \) (4.8 × 10⁻⁴ M⁻¹) and a Δ\( H \) of −11.2 kcal mol⁻¹. Analog 4 shows a Δ\( H \) of −14.6 kcal mol⁻¹ and a 2-fold reduction in \( K_a \) (5.6 × 10⁻⁴ M⁻¹). These results suggest substantial binding of the 3-OH of the α(1→3) arm of 1 with DGL and possible binding of the 4-OB on the same arm. The \( K_a \) values derived from the ITC measurements agree with hemagglutination inhibition studies, which show affinities of DGL for both analogs (2.5 × 10⁻⁵ M⁻¹ and 1.1 × 10⁻⁵ M⁻¹, respectively), relative to 1. The Δ\( H \) values of 10 and 11 are −14.8 kcal mol⁻¹ and −12.8 kcal mol⁻¹, respectively. These data suggest the involvement of the 2- and 4-OH of the central Man residue of 1 in binding to DGL.

**Binding of Tetradeoxy Analog of 15**—The α(1→3),3-deoxy, “core”2,4-deoxy analog (15) (Fig. 1) was synthesized, and its thermodynamic binding parameters were determined for DGL and ConA. Table I shows that DGL binds to 15 with 3-fold greater affinity than MecMan but 80-fold less than 1. Δ\( H \) for 15 is −9.9 kcal mol⁻¹. Thus, 15 possesses the lowest \( K_a \) and Δ\( H \) values of the deoxy analogs of 1 in Table I, consistent with the four missing hydroxyl groups of 15 in binding.

Table II shows corresponding data for ConA binding to 15. Δ\( H \) for 15 is −8.9 kcal mol⁻¹, and \( K_a \) is only slightly greater than that of MecMan. Thus, ConA and DGL show large reductions in their respective \( K_a \) and Δ\( H \) values for 15 relative to 1.

**Comparison of Thermodynamic Data for DGL and ConA Binding to Deoxy Analogs of 1**—ITC data for ConA binding to deoxy analogs 2–14 have previously been reported (8) and are shown in Table II for comparison. ITC data for ConA binding to tetradeoxy analog 15 determined in the present study are also shown in Table II. A comparison of the thermodynamic data in Table I for DGL and in Table II for ConA show similarities and
differences. Data for the deoxy analogs of the ω(1–3) arm of 1 show a lack of binding of both lectins to the 2- and 6-OH groups. On the other hand, reductions in $K_a$ and $\Delta H$ values for the ω(1–3)deoxy (3) analog provide evidence for interactions of the 3-OH group of 1 with both DGL and ConA. The ω(1–3)deoxy analog (4) shows a loss in $-\Delta H$ (2.4 kcal mol$^{-1}$) and a $-4$-fold reduction in $K_a$ for ConA binding and a loss in $\Delta H$ of 1.6 kcal mol$^{-1}$ and a 2-fold reduction in $K_a$ for DGL binding, indicating binding of the 4-OH on the ω(1–3) arm of 1 to ConA and to DGL.

Thermodynamic data for the “core”2- (10) and 4-deoxy (11) analogs of the central Man residue are similar for DGL and ConA, indicating binding of both lectins to these sites on 1.

ITC data for deoxy analogs 7–9 of the ω(1–6) arm of 1 indicate binding of the 3-, 4-, and 6-OH groups to both lectins (Tables I and II). However, unlike ConA, the data for deoxy analog 6 indicate binding of the 2-OH to DGL. The fact that DGL binds to the 3-, 4-, and 6-hydroxyls on the ω(1–6) Man residue of 1, which is similar to ConA, suggest that, like ConA (16), the ω(1–6) Man residue of 1 binds to the so-called mannosaccharide site of DGL. However, DGL shows average differences of about $-6.1$ kcal mol$^{-1}$ in the $\Delta H$ values for 7–9 relative to 1, which is nearly twice as great as the average $\Delta H$ of about $-3.2$ kcal mol$^{-1}$ for ConA binding to these analogs. The average loss in $\Delta G$ values for DGL and ConA binding to 7–9 is also greater for the former.

ITC data for tetradeoxy analog 15 show similar losses in $\Delta H$ for DGL (6.3 kcal mol$^{-1}$) (Table I) and ConA (5.8 kcal mol$^{-1}$) (Table II), consistent with the loss of the four hydroxyl groups in binding to both lectins.

Nonlinearity of the $\Delta H$ and $\Delta G$ Values of the Individual Hydroxyl Groups of 1 and Differences in the Thermodynamics of Binding of DGL and ConA to 1—The thermodynamic data for ConA in Table II together with the x-ray crystal structure of the lectin bound to 1 (16) have demonstrated that the $\Delta H$ and $\Delta G$ values for the monodeoxy analogs in Fig. 1 are nonlinear (8). For example, a nonlinear relationship in $\Delta H$ has been shown to be present in binding of the di- and trideoxy analogs of 1 to ConA (Table II) (8). $\Delta H$ for deoxy analog 12 is 4.1 kcal mol$^{-1}$ as compared with the sum of the $\Delta H$ values for corresponding monodeoxy analogs 3 and 10 of 5.0 kcal mol$^{-1}$. Likewise, $\Delta H$ for deoxy analog 13 is 5.0 kcal mol$^{-1}$ as compared with the sum of the $\Delta H$ values for corresponding monodeoxy analogs 3 and 11 of 6.3 kcal mol$^{-1}$. In the case of trideoxy analog 14, the $\Delta H$ value is 6.0 kcal mol$^{-1}$ as compared with the sum of the $\Delta H$ of 7.6 kcal mol$^{-1}$ for the corresponding monodeoxy analogs 3, 10, and 11. The same nonlinearity is also present in the $\Delta G$ values of these analogs (8).

In the present study, binding of ConA to tetradeoxy analog 15 results in a $\Delta H$ value of 5.8 kcal mol$^{-1}$, with respect to 1, compared with the sum of the $\Delta H$ values of 10.0 kcal mol$^{-1}$ for the four corresponding monodeoxy analogs, 3, 4, 10, and 11 (Table II). A similar result is also observed for DGL. The $\Delta H$ value for 15 binding to DGL is 6.3 kcal mol$^{-1}$, as compared with the sum of the $\Delta H$ values of $-11.4$ kcal mol$^{-1}$ for the four corresponding monodeoxy analogs, 3, 4, 10, and 11 (Table I). The $\Delta G$ values for 15 and the corresponding deoxy analogs also do not scale linearly (Table I).

The presence of nonlinear relationships in the $\Delta H$ and $\Delta G$ values for the deoxy analogs binding to DGL and ConA indicate other contributions to these thermodynamic parameters such as solvent and protein effects. Indeed, a recent study suggests a substantial contribution of solvent to the $\Delta H$ of binding of 1 to ConA (17). Thus, the observed $\Delta H$ and $\Delta G$ values in Tables I and II represent not only the loss of the hydrogen bond(s) involved but also differential solvation energies for the parent trimannoside relative to the monodeoxy analogs 2–11 and tetradeoxy analog 15. In addition, differential solvation and protein effects are expected for the respective DGL complexes with 1 and the deoxy analogs.

The x-ray crystal structure of the DGL-trimannoside complex determined in the second paper in this series (26) also provides confirmation of the nonlinear nature of the thermodynamic data presented in Table I of the present study.

**Binding Thermodynamics of Biantennary Complex Carbohydrate 16 to DGL**—Hemaggglutination inhibition experiments and affinity column chromatography have shown that DGL binds the biantennary complex carbohydrate 16 in Fig. 1 much more poorly than ConA (3). Longer chain analogs of 16 have also been shown to preferentially bind to ConA. The differential specificity of ConA and DGL for 16 has also recently been shown to be present in other members of the Diocleinae subtribe, which include ConA and DGL (5). Seven other Diocleinae lectins that have specificity and high affinities for trimannoside 1 have been shown by hemagglutination inhibition and microcolumn chromatography to have variable specificities for 16. However, the difference in binding specificities of ConA and DGL for 16 have been shown to be greatest in this family of proteins (5). Importantly, the ability of these nine Diocleinae lectins to induce histamine release from rat peritoneal mast cells (4) was shown to correlate with the relative affinities of the proteins for 16 (5). Thus, in order to elucidate the molecular basis for the biological activities of this family of lectins, an understanding of the relationship between their thermodynamic binding properties and their structures is required.

ITC data for DGL binding to 16 are shown in Table I. The $K_a$ value for DGL is $4.7 \times 10^4$ M$^{-1}$ as compared with a $K_a$ value of $1.2 \times 10^6$ M$^{-1}$ for 1. ITC data for ConA binding to 16 have been previously reported (15) and are shown in Table II for comparison. The $K_a$ value of ConA for 16 is $1.4 \times 10^6$ M$^{-1}$, which is nearly 3-fold greater than the $K_a$ value of $3.9 \times 10^5$ M$^{-1}$ for 1. This can be compared with the 26-fold weaker binding of DGL to 16 relative to 1. The $\Delta H$ values of the two lectins for 16 are very different, with $\Delta H = -10.6$ kcal mol$^{-1}$ for ConA and $\Delta H = -4.6$ kcal mol$^{-1}$ for DGL. These results indicate that although both lectins share high affinities and specificities for 1, they possess very different affinities and specificities for 16. The structural basis for this difference is investigated in the next paper in this series (26).

**Enthalpy-Entropy Compensation**—Enthalpy-entropy compensation plots have previously been observed for carbohydrate

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2 C. F. Brewer and T. S. Raju, unpublished results.
interactions with lectins (18, 19) and antibodies (20–22) and attributed to the unique properties of water (19). An enthalpy-entropy compensation plot for ConA binding to water (19). An enthalpy-entropy compensation plot for ConA binding to oligosaccharides 1–14 has been shown to be linear (8). Fig. 3 shows a plot of the \( -\Delta H \) versus \( -T\Delta S \) values in Table I for DGL binding to 1–11 and 15 (Table I). For comparison, corresponding data for ConA binding to 1–15 (Table II) is shown. The plot for DGL is linear (solid line) with a slope of 1.61 and a correlation coefficient 0.98 and is very similar to the plot for ConA (broken line), which also has a slope of 1.51 and a correlation coefficient of 0.95. This suggests very similar mechanisms of binding of the two lectins to 1 and its analogs. The enthalpy-entropy plots in Fig. 3 are similar to those reported earlier for other lectin-carbohydrate interactions in that their slopes are greater than unity (23, 24), in contrast to antibody-carbohydrate interactions where the slope is often less than unity (21, 22). A slope greater than unity means that the free energy of binding is predominantly driven by enthalpy, while a slope less than unity indicates dominant entropy contributions.

**Summary**—The present study provides a thermodynamic description of the binding of DGL to trimannoside 1 and its deoxy analogs and to complex carbohydrate 16. The results indicate binding of the 2-, 3-, 4-, and 6-hydroxyls of the \( \alpha(1–6) \) Man, the 2- and 4-OH groups of the “core” Man, and the 3- and 4-OH on the \( \alpha(1–3) \) Man of the lectin. The thermodynamic data for DGL binding to 1 and the deoxy analogs are compared with similar data for ConA (8). Differences in the thermodynamic data for deoxy analogs of the \( \alpha(1–6) \) Man of 1 are observed for the two lectins. The thermodynamic data for DGL binding to complex carbohydrate 16 indicate a different binding mechanism from that of ConA, as recently reported for several other members of the Diocleinae subtribe (5).

Nonlinear effects of the \( \Delta H \) and \( \Delta G \) values of the deoxy analogs binding to DGL (and ConA) indicate differential contributions of the solvent and protein to their binding and not direct measurements of the loss in hydrogen-bonding interactions.

The following paper (26) presents the x-ray crystal structure of DGL complexed to 1 and a comparison with the present thermodynamic data. In turn, the thermodynamic and structural data for DGL are compared with similar thermodynamic and structural data for ConA.

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