Thermodynamics of Monosaccharide Binding to Concanavalin A, Pea (Pisum sativum) Lectin, and Lentil (Lens culinaris) Lectin*

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Titration calorimetry measurements of the binding of methyl α-D-mannopyranoside (MeaMan), D-mannopyranoside (Man), methyl α-D-glucopyranoside (MeaGlu), and D-glucopyranoside (Glu) to concana- valin A (Con A), pea lectin, and lentil lectin were performed at 281 and 292 K in 0.01 M dimethylglutaric acid-NaOH buffer (pH 6.9) containing 0.15 M NaCl and Mn²⁺ and Ca²⁺ ions. The site binding enthalpies, ΔH, are the same at both temperatures and range from −28.4 ± 0.9 (MeaMan) to −16.6 ± 0.5 kJ mol⁻¹ (Glu) for Con A, from −26.2 ± 1.1 (MeaMan) to −12.8 ± 0.4 kJ mol⁻¹ (MeaGlu) for pea lectin, and from −16.6 ± 0.7 (MeaMan) to −8.0 ± 0.2 kJ mol⁻¹ (MeaGlu) for lentil lectin. The site binding constants range from 17 ± 1 × 10³ M⁻¹ (MeaMan to Con A at 281.2 K) to 230 ± 20 M⁻¹ (Glu to lentil lectin at 292.6 K) and exhibit high specificity for Con A where they are in the MeaMan:Man:MeaGlu:Glu ratio of 21:4:5:1, while the corresponding ratio is 5:2:1:5:1 for pea lectin and 4:2:2:1 for lentil lectin. The higher specificity for Con A indicates more interactions between the amino acid residues at the binding site and the carbohydrate ligand than for the pea and lentil lectin-carbohydrate complexes. The carbohydrate-lectin binding results exhibit enthalpy-entropy compensation in that ΔHb (kJ mol⁻¹) = −1.67 ± 0.05 × 10⁴ + (1.30 ± 0.12)T(K)ΔSb (J mol⁻¹K⁻¹).

Differential scanning calorimetry measurements on the thermal denaturation of the lectins and their carbohydrate complexes show that the Con A tetramer dissociates into monomers, while the pea and lentil lectin dimers dissociate into two submonomer fragments. At the denaturation temperature, one carbohydrate binds to each monomer of Con A and the pea and lentil lectins. Complexation with the carbohydrate increases the denaturation temperature of the lectin and the magnitude of the increases yield binding constants in agreement with the determinations from titration calorimetry.

Lectins are proteins which bind carbohydrates with a high degree of specificity. This makes them useful as probes for carbohydrate structure in various biological systems such as cell membranes, and as ideal model systems for elucidating protein-carbohydrate interactions. Although the legume lectins, concanavalin A (Con A), pea lectin, and lentil lectin are very similar in their structural properties, they exhibit different binding affinities for saccharide derivatives of the manno- pyranoside and glucopyranoside configuration (Goldstein et al., 1965; Debray et al., 1981). In solution, Con A below pH 5.8, pea lectin, and lentil lectin exist as dimers, composed of 25,000-30,000 molecular weight units (M₂), require Ca²⁺ and Mn²⁺ ions for carbohydrate binding, and have one carbohydrate binding site per monomer. X-ray analysis of the carbohydrate-lectin complexes, Con A-a-methyl-D-mannopyranoside (Derwenda et al., 1989) and pea lectin-methyl 3,6-di-O-(a-D-mannopyranosyl)-a-D-mannopyranoside (Rini et al., 1989), show that the carbohydrate binding sites are near the Ca²⁺ binding site which is adjacent to the Mn²⁺ binding site. Above pH 6.9, Con A exists as a tetramer, and there are structural differences between the monomers of Con A and of pea and lentil lectin (Einspahr et al., 1986). The monomers of Con A consist of a continuous polypeptide chain of 237 amino acid residues, while the monomers of pea and lentil lectin are each composed of two different polypeptide chains, one 6,000 Mₑ (α-chain) and one 18,000 Mₑ (β-chain) (Einspahr et al., 1986). The thermodynamics of the carbohydrate binding reaction is important in elucidating how these structural properties are related to the carbohydrate binding properties of the legume lectins.

In this investigation, titration calorimetry was employed to determine the thermodynamics of the carbohydrate-lectin binding reaction in terms of the binding constant (Kb), and changes in the free energy ΔGₘ, the binding enthalpy (ΔHₘ), and the binding entropy (ΔSₘ). The binding reaction between the lectin monomer (LM) and the carbohydrate (S) is

\[ LM + S \rightleftharpoons LM·S \]  

(Eq. 1)
in 0.150 M NaCl + 0.01 M 3,3'-dimethyl glutaric acid-NaOH (DMG) buffer with 0.001 M MnCl₂ and 0.001 M CaCl₂ at pH 6.90 ± 0.06. A few of the Con A and pea lectin reactions were performed at pH 7.4 with the 0.01 M DMG replaced by 0.02 M sodium pisosfate buffer and without the Mn²⁺ and Ca²⁺ salts. The titration calorimetry measurements were performed

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‡ The abbreviations used are: Con A, concanavalin A; Glu, D-glucopyranoside; DSC, differential scanning calorimetry; MeaMan, a-methyl-D-mannopyranoside; Man, D-mannopyranoside; MeaGlu, a-methyl-D-glucopyranoside; Tris, tris(hydroxymethyl)aminomethane.
The carbohydrates were \(\alpha\)-methyl-\(\alpha\)-mannopyranoside (MeaMan), \(\alpha\)-methyl-\(\beta\)-mannopyranoside (Man), \(\alpha\)-methyl-\(\beta\)-glucopyranoside (MeoGlu), and \(\alpha\)-glucopyranoside (Glu). In addition, differences in the structural stabilities, particularly the effect of carbohydrate binding on the thermal stabilities of the legume lectins, were investigated by differential scanning calorimetry (DSC). Previous DSC studies on the unfolding properties of Con A in the presence of carbohydrate ligands (Zahnley, 1981; Borrebaeck and Mattiasson, 1979) have shown that the presence of the ligand increases the thermal stability of the lectin.

**EXPERIMENTAL PROCEDURES**

**Materials**—Con A, lentil lectin, 3,3′-dimethylglutaric acid (DMG), Tris, and the monosaccharides were obtained from Sigma and used without any further purification. Pea lectin was prepared and purified to a purity >95% as described previously (Bhattacharyya et al., 1985). Some samples of lentil lectin were also prepared and purified by a similar procedure (Bhattacharyya et al., 1985). The specifications from Sigma stated that a 7% polyacrylamide electrophoresis gel for Con A with \(\beta\)-alanine buffer (pH 4.5) exhibited one major and two faint minor bands and for lentil lectin with Tris-glycine buffer (pH 8.3) exhibited two diffuse major bands and one faint minor band. The total monomer or major bands for lentil lectin correspond to the two \(\alpha\)-isoelectin dimers of lentil lectin, A and B, each with \(M_r = 49,000\). The binding properties of the isolectins are identical and isolectin B contains only 4 more lysine residues per monomer which accounts for their different electrophoretic properties (Howard et al., 1971).

Since the binding properties are identical, the lentil lectin will be considered as consisting of one species as was done in earlier studies (Bhattacharyya et al., 1985). A Pharmacia Phast native electrophoresis gel, with 0.1 M Tris-HCl buffer (pH 6.9) containing 0.2% bromophenol blue and 20% glycerol exhibited one major band for pea lectin and lentil lectin and one major band and a weak band for Con A. The glyceral, hydroxyproline, glycine, bromphenol blue, NaCl, MnCl₂, CaCl₂, and NaOH were of reagent quality.

**Preparation and Analysis of Solutions**—The lectin solutions were prepared in the appropriate buffer by weight, dialyzed overnight in a large volume of the same buffer, and centrifuged to remove any insoluble lectin. The DMG buffer was prepared by adding dropwise aliquots of concentrated NaOH solution to 0.01 M 3,3′-Dimethylglutaric acid in 0.15 M NaCl to achieve the desired pH as determined by an Orion 811 pH meter equipped with a Corning EXL glass electrode. The phosphate buffer was prepared as described previously (Schwarz et al., 1991). The best least-squares fit of the two-state transition model, A \(\leftrightarrow\) B, was then fitted via a nonlinear least squares minimization method (Wiseman et al., 1989) to the total ligand concentration, \(X\), using the following equation (Yang, 1990),

\[
Q_t = nM_t\Delta H_r/nM_t + 1/nM_t = 1/nK_bM_t
\]

\[
\Delta G_r = -\Delta H_r - T\Delta S_r
\]

\[\Delta H_r = nRT\ln[\text{K}_r]\]

where \(n\) is the number of moles, \(T\) is the absolute temperature, and \(R = 8.3151\) J mol\(^{-1}\) K\(^{-1}\).

**DSC Measurements**—DSC measurements were performed with a DSC 7500 DSC apparatus using a scan rate of 20°C min\(^{-1}\) and vaporizing nitrogen as purge gas (Schwarz et al., 1991). The best least-squares fit of the two-state transition model, A \(\leftrightarrow\) B, was then fitted via a nonlinear least squares minimization method (Wiseman et al., 1989) to the total peak height (\(C_m\)) to the peak area (\(A_r\)) as shown

\[
\Delta H_r = mRT\Delta C_p^{\text{m}}/A_r
\]

where \(n = 1, m = 4.00\) and for \(n = 4, m = 9.01,\) and \(T_r\) is the temperature of the peak maximum. The transition temperature, \(T_m\), is the temperature at half the peak area and the calorimetric enthalpy per peak area per number of moles of lectin monomer in the DSC cell.

**RESULTS**

**Titration Calorimetry Measurements**—The results of a typical titration calorimetry measurement, which consisted of adding 5-µl aliquots of 7.0 ± 0.2 mM MeoMan to 0.26 ± 0.01 mM Con A in DMG buffer at pH 6.9 and 293.2 K, are shown in Fig. 1. The results exhibit a monotonic decrease in the exothermic heat of binding from an initial heat of \(-0.728\) mJ with each addition. A plot of the total heat released as a function of total ligand concentration is shown in Fig. 2. Results of the best fits of Equation 2 to the slopes of similar plots for the carbohydrate ligands yield the \(K_t\) and \(\Delta H_r\) values presented in Tables I–III. Monomer concentrations of the lectin were used in the fitting procedure; the average tetramericities from these fits were close to one. The results were independent of lectin concentration (≤1 mM) and ligand concentration. The enthalpy of the carbohydrate dilution was negligible since no heat was observed upon the addition of 10 µl of 30 mM carbohydrate solution into the buffer solution.

As can be seen in Tables I–III, the binding reactions for Con A, pea lectin, and lentil lectin are all enthalpically driven. The enthalpies over the temperature range 281.2–298.2 K are the same indicating very little heat capacity change results from the binding reaction. With the exception of the Man-Con A titrations at pH 7.4 and 298.5 K and Man-pea lectin titrations at pH 7.4 and 257.5 K, the binding enthalpies are

A native electrophoresis gel was run on the native and denatured samples of the legume lectins in 0.1 M Tris buffer containing 0.2% bromophenol blue and 20% glycerol at pH 6.8. The electrophoresis gels were performed at 15°C in a Pharmacia Phast System with native buffer strips.

**Catalytic Titrations**—The calorimetric titrations of the carbohydrate ligand solutions into the lectin solution were performed with a Microcal Omega titration calorimeter as described previously (Schwarz et al., 1991). Briefly, 4–10-µl aliquots of the 5–30 mM ligand solution were added via a rotating stirrer-syringe to the 0.1–1 mM protein solution contained in a 1.38-ml cell. The additions were 3 min apart to allow the exothermic heat peak accompanying each addition to return to the base line prior to the next addition. This was continued until the last titration peak was less than 25% of the initial peak. The total heat, \(Q_t\), was then fitted via a nonlinear least squares minimization method (Wiseman et al., 1989) to the total ligand concentration, \(X_t\), using the following equation (Yang, 1990),
Thermodynamics of Monosaccharide Binding to Lectins

FIG. 1. Titration calorimeter results from adding 21 5-μl aliquots of 7.0 ± 0.2 mM MeM to 0.26 ± 0.01 mM Con A in DMG buffer at pH 6.9 and 293.2 K.

FIG. 2. A plot of the total heat released as a function of total ligand concentration for the titration shown in Fig. 1. The solid line is the result of the best least squares fit of the data to Equation 2.

also independent of pH from 6.9 to 7.4 for Con A and pea lectin and from pH 5.0 to 6.9 for MeaMan binding to Con A. The literature values (Ambrosino et al., 1987) of $K_b = 2.5 \pm 0.4 \times 10^3$ M$^{-1}$ and $\Delta H_b = 18 \pm 1$ kJ mol$^{-1}$ at pH 4.5 and 298.15 K for MeaGlu are the same as the corresponding average values at pH 6.9 in Table I.

The binding constants in going from MeaMan binding to Glu binding at room temperature decrease by a factor of 21 for Con A, a factor of 5, for pea lectin, and a factor of 4 for lentil lectin. In Table I, the binding constants close to room temperature are in the order $21 \pm 2.3.8 \pm 0.4:4.8 \pm 0.7:1.0$ for MeaMan:Man:MeaGlu:Glu which is close to the order of the binding affinities of 16:4:4:1 from inhibition studies (Debray et al., 1981). In Table II, the corresponding order for pea lectin is $4.8 \pm 0.5:2.2 \pm 0.1:1.5 \pm 0.1$ at room temperature which is close to 4:2:1, the order of the binding affinity from inhibition studies, (Debray et al., 1981). For lentil lectin, the order is $3.7 \pm 0.4:2.2 \pm 0.2:2.0 \pm 0.2:1.0$ which is the same as 4:2:2:1, the order of binding affinity from the inhibition studies (Debray et al. 1981). The relative affinities for these lectins as obtained by titration calorimetry are in general agreement with the inhibition studies, which are based on the minimum amount of ligand required to inhibit agglutination of human red blood cells. In addition, the binding constants for each ligand decrease in the order Con A > pea lectin > lentil lectin.

The binding enthalpies for the legume lectins increase by almost a factor of three from MeaMan-Con A to Glu-lentil lectin binding. For Con A, the binding enthalpies increase in the order of MeaMan < Man < MeaGlu < Glu, while for pea and lentil lectin the order with regard to Glu > MeaGlu is reversed. For each ligand, the enthalpies increase in the order Con A < pea lectin < lentil lectin. The entropy changes follow the same trends as the binding enthalpies.

DSC Measurements—Typical DSC scans of Con A, pea lectin, and lentil lectin in 0.01 M DMG buffer containing Mn$^{2+}$, Ca$^{2+}$, and 0.150 M sodium chloride at pH 6.90 ± 0.05 are shown in Fig. 3. The concentrations of the Mn$^{2+}$ and Ca$^{2+}$ ions were each 1 mM in the pea and lentil lectin solutions and 100 mM each in the Con A solutions. Lower concentrations of the ions in the Con A solutions produced an additional smaller, lower temperature transition peak (not shown) with a transition temperature, $T_m$, close to that of the less thermally stable metalless form of Con A, apoconcanavalin A (Schwarz, 1993). The metalless forms of pea and lentil lectin...
Table I

Thermodynamic parameters of the binding of carbohydrates to concanavalin A in 0.15 M sodium chloride solution and 0.01 M dimethylglutaric-sodium hydroxide buffer below pH 7 and 0.02 M sodium phosphate buffer at pH 7.4

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>pH</th>
<th>Temperature</th>
<th>K</th>
<th>(-\Delta_{G}^0)</th>
<th>(-\Delta_{H}^0)</th>
<th>(\Delta_{S}^0)</th>
<th>Determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl (\alpha-D)-mannopyranoside</td>
<td>6.9</td>
<td>282.3 ± 0.8</td>
<td>3.28 ± 0.01</td>
<td>19.0 ± 0.3</td>
<td>25.5 ± 1.2</td>
<td>22.4 ± 4</td>
<td>3</td>
</tr>
<tr>
<td>7.4</td>
<td>287.6 ± 0.2</td>
<td>2.69 ± 0.05</td>
<td>18.9 ± 0.0</td>
<td>22.2 ± 0.2</td>
<td>11.7 ± 2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>6.9</td>
<td>291.9 ± 0.9</td>
<td>1.8 ± 0.16</td>
<td>18.3 ± 0.2</td>
<td>27.3 ± 0.5</td>
<td>31.2 ± 4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>7.4</td>
<td>298.3 ± 0.1</td>
<td>1.65 ± 0.14</td>
<td>18.4 ± 0.2</td>
<td>24.7 ± 0.3</td>
<td>21.0 ± 0.2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>D-Mannopyranoside</td>
<td>6.9</td>
<td>282.5 ± 0.0</td>
<td>4.15 ± 0.19</td>
<td>19.6 ± 0.1</td>
<td>19.4 ± 1.9</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>7.4</td>
<td>287.6 ± 0.1</td>
<td>2.99 ± 0.10</td>
<td>19.3 ± 0.1</td>
<td>17.9 ± 0.9</td>
<td>5 ± 1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>6.9</td>
<td>292.4 ± 0.3</td>
<td>2.71 ± 0.13</td>
<td>19.2 ± 0.1</td>
<td>18.1 ± 0.4</td>
<td>4 ± 2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>5.8</td>
<td>297.6 ± 0.1</td>
<td>2.23 ± 0.08</td>
<td>19.1 ± 0.1</td>
<td>25.2 ± 1.3</td>
<td>21 ± 4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>D-Glucopyranoside</td>
<td>6.9</td>
<td>282.6 ± 0.5</td>
<td>0.82 ± 0.08</td>
<td>15.8 ± 0.2</td>
<td>16.1 ± 2.2</td>
<td>1 ± 8</td>
<td>4</td>
</tr>
<tr>
<td>6.5</td>
<td>292.3 ± 0.1</td>
<td>0.56 ± 0.04</td>
<td>15.4 ± 0.2</td>
<td>17.1 ± 1.4</td>
<td>6 ± 5</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

a At pH 6.9, the solution contained 1 mM each of MnCl₂ and CaCl₂. The concentrations of concanavalin A ranged from 0.18 to 0.43 mM, while the carbohydrate concentration ranged from 5.0 to 32.0 mM.

Table II

Thermodynamic parameters of the binding of carbohydrates to pea lectin in 0.15 M sodium chloride solution and 0.01 M dimethylglutaric-sodium hydroxide buffer below pH 7 and 0.02 M sodium phosphate buffer at pH 7.4

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>pH</th>
<th>Temperature</th>
<th>K</th>
<th>(-\Delta_{G}^0)</th>
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<th>Determinations</th>
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</thead>
<tbody>
<tr>
<td>Methyl (\alpha-D)-mannopyranoside</td>
<td>6.9</td>
<td>282.3 ± 0.8</td>
<td>3.28 ± 0.01</td>
<td>19.0 ± 0.3</td>
<td>25.5 ± 1.2</td>
<td>22.4 ± 4</td>
<td>3</td>
</tr>
<tr>
<td>7.4</td>
<td>287.6 ± 0.2</td>
<td>2.69 ± 0.05</td>
<td>18.9 ± 0.0</td>
<td>22.2 ± 0.2</td>
<td>11.7 ± 2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>6.9</td>
<td>291.9 ± 0.9</td>
<td>1.8 ± 0.16</td>
<td>18.3 ± 0.2</td>
<td>27.3 ± 0.5</td>
<td>31.2 ± 4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>7.4</td>
<td>298.3 ± 0.1</td>
<td>1.65 ± 0.14</td>
<td>18.4 ± 0.2</td>
<td>24.7 ± 0.3</td>
<td>21.0 ± 0.2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>D-Mannopyranoside</td>
<td>6.9</td>
<td>282.0 ± 0.3</td>
<td>1.67 ± 0.2</td>
<td>17.4 ± 0.3</td>
<td>25.5 ± 2.1</td>
<td>29.7 ± 3</td>
<td>3</td>
</tr>
<tr>
<td>7.4</td>
<td>287.3 ± 0.1</td>
<td>1.02 ± 0.14</td>
<td>16.8 ± 0.3</td>
<td>22.2 ± 0.9</td>
<td>29.3 ± 3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6.9</td>
<td>292.3 ± 0.9</td>
<td>0.87 ± 0.02</td>
<td>16.5 ± 0.1</td>
<td>24.8 ± 0.8</td>
<td>29 ± 3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>7.4</td>
<td>297.5 ± 0.1</td>
<td>0.79 ± 0.01</td>
<td>16.5 ± 0.0</td>
<td>17.7 ± 3.3</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>D-Glucopyranoside</td>
<td>6.9</td>
<td>281.5 ± 0.4</td>
<td>0.94 ± 0.05</td>
<td>16.0 ± 0.1</td>
<td>12.4 ± 0.7</td>
<td>-13 ± 3</td>
<td>4</td>
</tr>
<tr>
<td>7.4</td>
<td>285.2 ± 0.3</td>
<td>0.77 ± 0.04</td>
<td>16.0 ± 0.1</td>
<td>10 ± 1.7</td>
<td>-15 ± 2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>6.9</td>
<td>291.4 ± 0.4</td>
<td>0.61 ± 0.03</td>
<td>15.5 ± 0.1</td>
<td>13.1 ± 0.1</td>
<td>-8 ± 1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6.8</td>
<td>296.2 ± 0.2</td>
<td>1.22 ± 0.03</td>
<td>15.5 ± 0.1</td>
<td>13.1 ± 0.1</td>
<td>-8 ± 1</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

a At pH 6.9, the solution contained 1 mM each of MnCl₂ and CaCl₂. The concentrations of pea lectin ranged from 0.18 to 0.56 mM, while the carbohydrate concentration ranged from 12 to 27.0 mM.

Average values at pH 6.9.

are unstable at this pH (Bhattacharyya et al., 1985) since dialysis of lentil lectin against EDTA in 1 M acetic acid to remove Mn⁺⁺ also leads to dissociation of the lectin into its \(\alpha\) and \(\beta\) chains (Fliegerova et al., 1974). Upon completion of the scan, a precipitate was observed in the cells. Upon rescanning the solutions, the transitions did not reappear, indicating that the transitions are irreversible. All the peaks exhibit an asymmetry which corresponds to a protein undergoing dissociation as it unfolds. As shown by the solid lines in Fig. 3, the peaks are best fitted to a two-state transition of a protein unfolding and dissociating into four subfragments which may or may not be identical in size as follows.

\[ A \leftrightarrow B' + B'' + B''' + B'''' \quad \text{or} \quad 4B \quad (\text{Eq. 6}) \]

Con A exists as a tetramer at pH 6.9, while pea and lentil lectins exist as dimers at this pH. Apparently, Con A denatures into its monomeric units, while pea and lentil lectin undergo dissociation into their \(\alpha\) and \(\beta\) chains. This is further substantiated by a native electrophoresis gel run on the native and denatured solutions of the three lectins. A native electrophoresis gel of the denatured pea and lentil solutions exhibited only two major bands well below the middle of the gel where the bands for the wild type lectins appear. These bands are presumably the \(\alpha\) and \(\beta\) chains. Samples of the denatured Con A solutions did not exhibit these bands.

There is a slight transition temperature dependence on the scan rate as shown in Table IV. A maximum increase in the scan rate from 8 to 55 K h⁻¹ increases the transition and maximum temperatures of the denaturation transitions by 3 ± 1 K but had no effect on the shapes of the transitions. This
shift was determined by comparison with DSC measurements on the reversible unfolding transition of lysozyme in 0.2 M HCl-glycine-buffered solutions (pH 3.8) which also showed a slight transition temperature increase (1 K) at the higher scan rate when scanned under the same conditions. Since this temperature increase is small and the shapes of the transitions are the same, the equilibrium two-state transition model was applied to these transitions instead of the irreversible model of Sanchez-Ruiz (1989). This analysis of the irreversible DSC transitions in terms of a thermodynamic model is based on treating the transitions as a sequence of two processes; the reversible unfolding of the protein described by the unfolding temperature and the calorimetric enthalpy followed by a slower irreversible process such as aggregation. This approach yields results for the overall process that are the same as for the reversible process (Manley et al., 1985).

Values of the transition temperature (Tm), the calorimetric enthalpy (∆Hm), and the van’ t Hoff enthalpy (∆Hv) are presented in Table IV for each of the lectins at different pH values. The type of transition expressed in Equation 6 is also supported by the increase with lectin concentration in both the transition temperature and the temperature at the peak maximum (Tg) as shown below. The van’t Hoff enthalpy is in terms of moles of tetramer for Con A and moles of dimer for pea and lentil lectin. The calorimetric enthalpy is in terms of moles of oligomer, tetramer for Con A and dimer for the pea and lentil lectins. The calorimetric enthalpy is in terms of moles of oligomer, tetramer for Con A and dimer for the pea and lentil lectins. Also presented in Table IV is the ratio of the calorimetric enthalpy to the van’t Hoff enthalpy determined from the fit of the two-state transition model. The ratio is greater than one for Con A and close to one for pea and lentil lectin. The denaturation of the pea and lentil lectins follow the two-state model, while the higher ratios for Con A indicate the presence of an intermediate state, perhaps the metal-less form of Con A, apoconcanavalin A.

Fukada et al.’s (1983) treatment for the denaturation peak temperature dependence on protein concentration of an oligomer dissociating into its monomer units can be extended to a protein dissociating into four subunits upon denaturation,

\[(AA^\prime)_L \leftrightarrow 2B + 2B^\prime\]  
(Eq. 7)

where A is the native form of one fragment, A’ is the other fragment, and B is the unfolded states of these fragments. It can be shown that

\[\ln[(AA^\prime)_L] = -\Delta H_s(S)/[RT_p(n - 1)] + \text{constant}\]  
(Eq. 8)

where n = total number of moles of denatured species, 4. Plots of Equation 8 are shown in Fig. 4. In Table IV, the values of ∆Hv determined from Equation 8, ∆Hv(S), are close to the calorimetric enthalpies determined when n = 4 for Con A and pea and lentil lectin. It should be emphasized that the temperatures at the peak maxima are independent of the fits of the two-state transition model to the data and, thus, the overlap of ∆Hv with ∆Hv(S) is independent confirmation that denaturation of the pea and lentil dimers results in dissociation of their monomers and denaturation of the Con A tetramer results in dissociation into its monomers.

In Table IV, there is little dependence of the thermodynamic parameters on pH down to pH 5 for Con A and pea lectin, while lentil lectin results exhibit a decrease in Tm from pH 6.9 to 5.0 along with corresponding decrease in the calorimetric and van’t Hoff enthalpies. Lentil lectin is similar to globular proteins which exhibit decrease in the transitional enthalpies and added ∆Hv(S), while Con A and pea lectin exhibit a decrease in both Tm and ∆Hv(S). Lack of pH dependence of transition temperature for Con A and pea lectin indicates the absence of any proton transfer as the unfolded form. The thermal stability of the pea and lentil lectins are the same but less than that of Con A which unfolds at higher temperatures.

In the presence of saturating amounts of carbohydrate ligands, the transition temperatures and calorimetric enthalpies, and the van’t Hoff enthalpies exhibit increases, while the calorimetric to van’t Hoff enthalpy ratios remain the same. The increase in both Tm and Tg with ligand concentration arises from binding of the ligand to the lectin only in the folded form. The denaturation transition in the presence of bound ligand can be expressed as follows,

\[(AA^\prime)_L \leftrightarrow 2B + 2B^\prime + mL\]  
(Eq. 9)

and at constant lectin concentration,

\[\ln[L] = -\Delta H_s(L)/[RT_p(n - 1)] + \text{constant}\]  
(Eq. 10)

The best fits of ln[L] to 1/Tg yield van’t Hoff enthalpies close to the calorimetric enthalpies for m = 4 for the Con A complex and m = 2 for the pea and lentil complexes. Thus, one carbohydrate binds to each of the four monomer Con A units and to each of the two pea and lentil lectin monomer units.
FIG. 3. a, DSC scan of a 0.5-g sample of 0.53 mM Con A in DMG buffer containing 100 mM each of Ca\textsuperscript{2+} and Mn\textsuperscript{2+} ions at a scan rate of 13 K h\textsuperscript{-1}. The solid lines are the best least squares fits of the DSC data to the A ↔ 4B two state transition model. b, DSC scan of a 0.5-g sample of 0.28 mM pea lectin in DMG buffer containing 1 mM each of Ca\textsuperscript{2+} and Mn\textsuperscript{2+} ions at a scan rate of 13 K h\textsuperscript{-1}. The solid lines are the best least squares fits of the DSC data to the A ↔ 4B two state transition model. c, DSC scan of a 0.5-g sample of 0.28 mM lentil lectin in DMG buffer containing 1 mM each of Ca\textsuperscript{2+} and Mn\textsuperscript{2+} ions at a scan rate of 13 K h\textsuperscript{-1}. The solid lines are the best least squares fits of the DSC data to the A ↔ 4B two state transition model.
It has been shown (Schwarz, 1988) that the increase in the transition temperature with ligand concentration at [L] >> [protein] depends on the association constant and that the association constant at the denaturation temperature can be determined from the increase in the denaturation and ligand concentration as shown,

\[ K_b(T_m) = \exp(\Delta H_b/1/T_m(0) - 1/T_m/R)(1 - [L]) \]  

(11)

where \( \Delta H_b \) is the denaturation enthalpy, \( T_m(0) \) is the denaturation temperature in the absence of ligand, and \([L]\) is the total ligand concentration. Using the calorimetric enthalpies in Table V, and \( T_m(0) = 362.4 \pm 0.1 \) K for Con A, 348.6 \pm 0.1 K at pH 6.9 and 345.9 K at pH 7.4 for pea lectin, and 346.5 \pm 0.1 K for lentil lectin, values for the binding constant at the transition temperature, \( K_b(T_m) \), were determined. In Table V, these values are compared to the values for \( K_b \) determined from extrapolating the titration calorimetry results in Tables I–III using \( K_b(T_m) = K_b(T)\exp(\Delta H_b(1/T - 1/T_m)/R) \). The \( T_m(0) \) values used in the determination of \( K_b(T_m) \) yield the most consistent values for the \( K_b \) for each carbohydrate and were within experimental error of the transition temperatures of the ligand free lectin. The results are shown in Table V in terms of the ratio \( K_b(K_b(DSC)) \) which is close to one, indicating that the titration calorimetry results agree with the DSC results for the determination of \( K_b \). The DSC results are also independent of pH from 6.9 to 7.4 and of scan rate as shown in Table V for the pea lectin. This lack of pH dependence for the binding constant was also observed with titration calorimetry at room temperature.

There are also some additional changes in the thermodynamic unfolding parameters in the presence of carbohydrate ligands. The calorimetric enthalpies are higher but do overlap with the calorimetric enthalpies in the absence of ligand for Con A and lentil lectin, while they are, however, higher by about 45 \pm 11\% for the pea lectin. The higher calorimetric enthalpies, especially for the pea lectin, can reflect a positive dependence of the transition enthalpy on temperature as observed with the globular proteins.

**DISCUSSION**

Earlier interpretations (Goldstein et al., 1965; Poretz and Goldstein, 1970; Bhattacharyya and Brewer, 1988) of the binding mechanism of carbohydrates to the legume lectins have relied on comparisons of the binding constants. To elucidate the binding mechanism, however, requires a more complete picture of the thermodynamics of the binding process. The loss in free energy of the ligand-lectin complex upon its formation involves not only enthalpy changes but also entropy changes which can be quite significant as shown by the titration calorimetry results. For example, the affinity of Glu binding to lentil lectin, \( \Delta G_b \) = \( -13.2 \pm 0.2 \) kJ mol\(^{-1}\), consists of a decrease in the enthalpy, \( \Delta H_b \) = \( -9.7 \pm 0.9 \) kJ mol\(^{-1}\), and a substantial increase in the entropy of the system, \( T\Delta S_b \) = \( 3.9 \pm 1.2 \) kJ mol\(^{-1}\), upon binding. Changes in the entropy can be interpreted in terms of differences in the interactions between the solvent and lectin and between the ligand and lectin. These interactions explicitly involve hydrogen bonding, electrostatic, and van der Waals interactions which can be identified from knowledge of the positioning of the ligand at the lectin binding site and from energy minimization calculations (Imberty et al., 1991). It is, however, difficult to explicitly identify the sources of the entropy contributions to the binding reaction. In fact, earlier interpretations of the binding affinities (Bhattacharyya and Brewer, 1988) ignored the entropic contributions and relied on describing changes in \( \Delta G_b \) in terms of pair-wise interactions between the carbohydrate OH groups and the amino acid residues at the binding site. As can be seen with the Glu-lentil lectin interactions, neglecting entropy effects can substantially alter the estimation of the binding affinity, \( \Delta G_b \).

The thermodynamics of the binding reaction presented in Tables I–III show that the changes in enthalpy and entropy upon binding appear to be compensatory. This "compensatory" effect is shown in Fig. 5 where \( \Delta H_b \) values for all the lectins are plotted as a function of \( T\Delta S_b \) at 292 K. The compensatory correlation,

\[ \Delta H_b = -1.67 \pm 0.06 \times 10^4 + (1.30 \pm 0.12)T\Delta S_b \]  

(12)

is quite good in that almost all the data lie within three standard deviations of \( \Delta S_b \) predicted from Equation 12 and that \( d(\Delta H_b)/d(T\Delta S_b) \) is close to 1. This correlation not only applies to the binding of each carbohydrate to a specific lectin but extends to the binding of the carbohydrate ligands to all three lectins. Similar enthalpy-entropy compensation with the slope of \( T\Delta S_b \) close to 1 has been observed in many protein-ligand interactions where the experimental conditions are fixed and the ligand structure is varied (congener series) (Lumry and Rajender, 1970; Belleau and Lavoie, 1968). This has been interpreted (Leffler and Grunwald, 1963) to mean that as the ligand becomes more tightly bound to the protein
Thermodynamics of Monosaccharide Binding to Lectins

(i.e. larger decrease in \(\Delta H_b\)), its rotational and vibrational degrees of freedom will become more restricted and, thus, the entropy will decrease. Another explanation (Eftink et al., 1983) for this compensation can arise from the assumption that the protein is in equilibrium between two different states, \(P_0 \leftrightarrow P_1\) and that the ligand binds to either state with different affinities to produce mixed \(P_0L\) and \(P_1L\) complexes. This coupling between the binding process and the transition between the two states results in enthalpy-entropy compensation. Since the observed heat capacity change upon carbohydrate binding is 0, the enthalpy change for the \(P_0 \leftrightarrow P_1\) is close to zero. Whether this enthalpy-entropy compensation

FIG. 4. a, a plot of ln[lectin concentration] vs. \(1/T_p\) for Con A. b, a plot of ln[lectin concentration] vs. \(1/T_p\) for pea lectin. c, a plot of ln[lectin concentration] vs. \(1/T_p\) for lentil lectin.
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Table V

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Ligand conc. / lectin conc.</th>
<th>$T_{m}$ (°C)</th>
<th>$\Delta H_{m}$ (kcal/mol)</th>
<th>$\Delta S_{m}$ (cal deg$^{-1}$/mol)</th>
<th>$\Delta H_{m}/\Delta S_{m}$ (kcal deg$^{-1}$/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con A</td>
<td>22–65/0.721</td>
<td>367.4–367.9</td>
<td>2882 ± 68</td>
<td>1574 ± 38</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Man</td>
<td>11–44/0.712</td>
<td>364.8–366.7</td>
<td>2784 ± 200</td>
<td>1530 ± 99</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>MeaGlu</td>
<td>18–55/0.712</td>
<td>365.5–367.8</td>
<td>2744 ± 120</td>
<td>1628 ± 51</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Glu</td>
<td>21–66/0.712</td>
<td>365.1–366.2</td>
<td>2392 ± 176</td>
<td>1673 ± 33</td>
<td>1.4 ± 0.1</td>
</tr>
</tbody>
</table>

Pea lectin

| MeaMan   | 19–74/0.165                 | 350.9–352.8  | 1696 ± 40                 | 1494 ± 31                         | 1.00 ± 0.05                                   |
| pH 7.4   | 21–44/0.286                 | 349.1–350.1  | 1522 ± 20                 | 1574 ± 60                         | 1.00 ± 0.05                                   |
| pH 7.4   | 23/0.016                    | 350.1 ± 0.2  | 1538 ± 28                 | 1704 ± 18                         | 0.95 ± 0.15                                   |
| Man      | 18–76/0.154                 | 350.2–351.8  | 1742 ± 139                | 1602 ± 11                         | 1.10 ± 0.25                                   |
| MeaGlu   | 21–69/0.165                 | 350.7–352.2  | 1494 ± 130                | 1554 ± 81                         | 1.05 ± 0.10                                   |
| Glu      | 18–76/0.150                 | 349.8–351.4  | 1670 ± 34                 | 1506 ± 74                         | 1.11 ± 0.10                                   |

Lentil lectin

| MeaMan   | 24–73/0.635                 | 350.0–351.2  | 1318 ± 128                | 1347 ± 73                         | 1.0 ± 0.05                                     |
| Man      | 24–76/0.635                 | 349.2–351.1  | 1200 ± 60                 | 1606 ± 41                         | 0.7 ± 0.1                                      |
| MeaGlu   | 20–75/0.635                 | 349.2–351.1  | 1226 ± 54                 | 1422 ± 57                         | 0.9 ± 0.1                                      |
| Glu      | 21–80/0.635                 | 348.6–350.5  | 1240 ± 80                 | 1463 ± 60                         | 0.9 ± 0.1                                      |

*The $\Delta H_{m}(L)$ values were determined from Equation 10 with $m = 4$ for the con A complexes and $n = 2$ for the pea and lentil complexes.

The values for $K_{s}$ (DSC) were determined from Equation 11, while those for $K_{s}$ were determined from the $K_{s}$ and $\Delta H_{m}$ values at room temperature (see text).

The scan rate was 45 K h$^{-1}$, while the usual scan rate was 13 K h$^{-1}$.

Fig. 5. A plot of $\Delta H_{m}$ vs. $\Delta S_{m}$ at room temperature for all the lectin complexes. The straight line is the best least squares fit of the data to a straight line.

extends to other carbohydrate ligands and lectins remains to be seen. This relationship shows that for monosaccharide binding to the legume lectins it is possible to estimate the enthalpy changes in the binding reaction from estimations of the enthalpy changes. The enthalpy changes can then be estimated from the pair-wise interactions and energy minimization methods such as the docking of MeaMan and MeaGlu to Con A as described by Imberty et al. (1991). Furthermore, enthalpy-entropy compensation shows that, again, changes in $\Delta G_{m}^{0}$ are not as specific a criterion of the binding interactions as is $\Delta H_{m}$ since $\Delta G_{m}^{0}$ shows less variation for the binding reactions.

Comparison of the binding constants among the three lectins show that in going from MeaMan to Glu, the specificity increases in the order Con A > pea lectin > lentil lectin. In comparing the D-mannopyranoside derivative results, there is little change in the entropy for Con A and pea lectin so that the increase in the binding affinity upon the addition of a methyl group results from an increase in the enthalpy, i.e. stronger interactions between the ligand and lectin. A comparison of the Man and MeaGlu results yields almost the same binding constant for each lectin which arises from the compensatory changes in the binding enthalpy and entropy, resulting in similar values for $\Delta G_{m}^{0}$. The only difference between Glu and Man is the positioning of the OH group on C2: in Glu it is equatorial, while in Man it is axial to the pyranose ring. This difference is significant in that it weakens substantially the binding interaction (enthalpy) for the D-glucopyranoside derivatives to all three lectins. From the x-ray crystallographic binding study of MeaMan to Con A, Derwenda et al. (1989) observed that this OH group is involved in water bridges to Ser-168 and Thr-226 or Asp-71 of an adjacent tetramer, so that in solution where the Con A tetramers are far apart it is not clear how this group affects the binding interactions (enthalpy).
MeaGlu does not interact with any of the amino acid residues in most of the binding configurations. In addition, Imberty et al. (1991) show that the C2-OH group interacts with the binding site (Leu-99 residue) only in the Man configuration. The decrease in \( \Delta H \) from the D-mannopyranoside to D-glucopyranoside derivatives can, thus, be attributed to the lack of interaction between C2-OH of the D-glucopyranoside derivatives and Con A.

The addition of a methyl group on the C1-OH group to form the \( \alpha \)-methyl derivatives enhances the binding enthalpies for the mannopyranosides, while decreasing the binding enthalpies for the pea and lentil lectin-glucopyranosides. This is likely the result of an inductive effect on the distribution of the charge density on the pyranoside ring since the methyl group can act as a slightly charge withdrawing group. For the mannopyranosides, while decreasing the binding in most of the binding configurations. In addition, Imberty et al. (1991) show that the C2-OH group interacts with the terminal mannopyranoside of the A-MeGlu does not interact with any of the amino acid residues of pea lectins, while decreasing the binding enthalpies for the pea and lentil lectin-glucopyranosides, its effect on the mannopyranosides, while decreasing the binding enthalpies for the pea and lentil lectin-glucopyranosides, while decreasing the binding enthalpies for the pea and lentil lectin-glucopyranosides.

The increase in carbohydrate binding specificity in going from Con A to lentil lectin can be attributed to less carbohydrate-amino acid interactions in the complexes of the pea and lentil lectins. Rini et al. (1989) identified hydrogen bond formation between pea lectin and only the C3-OH, C4-OH, and C5-OH groups of the terminal mannopyranoside of Con A. The DSC results show that at the denaturation temperature, the pea and lentil lectin dimers dissociate ultimately into sub-monomer fragments upon unfolding. Earlier DSC results (Zahnley, 1981) on Con A indicate that denaturation leads to demetallization since the denaturation temperature agrees with the observation that demetallization of pea lectin increases with metallization of the metalless form of Con A.

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The thermal stability of Con A is higher. This enhanced stability of Con A does not result from the increase metal concentration since there is very little temperature shift of the major transition peak upon going from 1 to 100 mM metal ion concentration for the Con A DSC scans. This lower stability may be attributed, again, to the less integrated structure of the pea and lentil monomers which readily dissociate in acid pH with EDTA or as these lectins absorb heat. Binding of the carbohydrates increases the thermal stability of the lectin and \( K_d \) at the denaturation temperature agrees with the titration calorimetry results at room temperature. Con A, pea, and lentil lectin exhibit single peak transitions which are indicative of globular-type proteins.

REFERENCES