Differential Solvation of “Core” Trimannoside Complexes of the *Dioclea grandiflora* Lectin and Concanavalin A Detected by Primary Solvent Isotope Effects in Isothermal Titration Microcalorimetry*

(Received for publication, July 2, 1998, and in revised form, September 3, 1998)

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The thermodynamics of binding of the Man/Glc-specific seed lectin from *Dioclea grandiflora* (DGL) to deoxy analogs of the “core” trimannoside, 3,6-di-O-(α-D-mannopyranosyl)-α-D-mannopyranoside was determined by isothermal titration microcalorimetry (ITC) in the first paper of this series (Dam, T. K., Oscarson, S., and Brewer, C. F. (1998) *J. Biol. Chem.* 273, 32812–32817). The data showed binding of specific hydroxyl groups on all three residues of the trimannoside, similar to that observed for ConA (Gupta, D., Dam, T. K., Oscarson, S., and Brewer, C. F. (1997) *J. Biol. Chem.* 272, 6388–6392). However, differences exist in the thermodynamics of binding of monodeoxy analogs of the α(1–6) Man residue of the trimannoside to the two lectins. The x-ray crystal structure of DGL complexed to the core trimannoside, presented in the second paper in this series (Rozwarski, D. A., Swami, B. M., Brewer, C. F., and Sacchettini, J. C. (1998) *J. Biol. Chem.* 273, 32818–32825), showed the overall structure of the complex to be similar to that of the ConA-trimannoside complex. Furthermore, the trimannoside is involved in nearly identical hydrogen bonding interactions in both complexes. However, differences were noted in the arrangement of ordered water molecules in the binding sites of the two lectins. The present study presents ITC measurements of DGL and ConA binding to the monodeoxy analogs of the trimannoside in hydrogen oxide (H\textsubscript{2}O) and deuterium oxide (D\textsubscript{2}O). The solvent isotope effects present in the thermodynamic binding data provide evidence for altered solvation of the parent trimannoside complexes at sites consistent with the x-ray crystal structures of both lectins. The results indicate that the differences in the thermodynamics of DGL and ConA binding to α(1–6) monodeoxy analogs of the trimannoside do not correlate with solvation differences of the parent trimannoside complexes.

In the first paper in this series (15), the thermodynamics of binding of the Man/Glc-specific seed lectin from *Dioclea grandiflora* (DGL)\textsuperscript{1} to deoxy analogs of the “core” trimannoside, 3,6-di-O-(α-D-mannopyranosyl)-α-D-mannopyranoside, was determined by isothermal titration microcalorimetry (ITC). The results showed evidence for the binding of the 2-, 3-, 4-, and 6-hydroxyl groups of the α(1,6) Man residue, the 3- and 4-hydroxyl groups of the α(1,3) Man residue, and the 2- and 4-hydroxyl groups of the central Man residue of the trimannoside. The ITC results are similar to those observed for the jack bean lectin, concanavalin A (ConA) (1), which also binds the trimannoside with high affinity; however, differences exist in the thermodynamics of binding of monodeoxy analogs of the α(1–6) Man residue of the trimannoside to the two lectins. The loss in the enthalpy of binding (ΔH) of these analogs to DGL, relative to the parent trimannoside, is nearly 3 kcal mol\textsuperscript{-1} greater than that for ConA (1). Furthermore, the loss in ΔH for DGL binding to the 2-deoxy α(1–6) analog is not observed for ConA binding to the analog (1).

In the second paper in this series, the x-ray crystal structure of DGL complexed to the parent trimannoside (16) shows that the overall structure of the complex is similar to that of the ConA-trimannoside complex (2). The average deviation in α-carbon position between the DGL tetramer in complex with the trimannoside and the ConA tetramer in complex with the trisaccharide is only 0.84 Å (16). Furthermore, the location and conformation of the bound trimannoside as well as its hydrogen bonding interactions are nearly identical in both lectin complexes. However, differences exist in the location of two loops outside of the respective binding sites containing residues 114–125 and residues 222–227. The latter residues effect the location of a network of hydrogen-bonded water molecules that interact with the trisaccharide. Differences in the arrangement of ordered water molecules in the binding site of the two lectins may account for their differences in the thermodynamics of binding to deoxy α(1–6) analogs of the trimannoside (15).

In the present paper, ITC studies of the binding of DGL and ConA to mono- and disaccharides, and to trimannoside 2-11 (Fig. 1) in hydrogen oxide (H\textsubscript{2}O) and deuterium oxide (D\textsubscript{2}O) are reported. The results show primary solvent isotope effects in the ΔH values of both lectins binding to the carbohydrates that correlate with the altered solvation

\footnotetext[1]{The abbreviations used are: DGL, seed lectin from *D. grandiflora*; ConA, concanavalin A, lectin from jack bean; ITC, isothermal titration microcalorimetry; Man, mannose; MeoMan, methyl α-D-mannopyranoside; MeoGlc, methyl α-D-glucopyranoside; Meo2dMan, methyl 2-deoxy-α-D-mannopyranoside.}

*This work was supported by NCI, National Institutes of Health (NIH), Department of Health, Education and Welfare, Grant CA-16054 and Core Grant P30 CA-13330 from the same agency (to C. F. B.) as well as NIGMS (NIH) Grant GM-43589 and the Wolfe-Welch Foundation (to J. C. S.). The NMR facility at AECOM was supported by NIH instrumentation Grant I-S10 RR02309 and National Science Foundation DMB-8413723. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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observed in the x-ray structures of the two lectins complexed to the parent trimannoside.

EXPERIMENTAL PROCEDURES

DGL was isolated from Dioclea grandiflora seeds obtained from northeastern Brazil (Albano Ferreira Martins Ltd., Sao Paulo, Brazil) as described previously (3). The concentration of DGL was determined spectrophotometrically at 280 nm using $A_{280}^\text{max} = 12.0$ at pH 7.2 and expressed in terms of monomer ($M_0 = 25,000$) (3). ConA was prepared from jack bean (Canavalia ensiformis) seeds (Sigma) according to the method of Agrawal and Goldstein (4). The concentration of ConA was determined spectrophotometrically at 280 nm using $A_{280}^\text{max} = 13.7$ at pH 7.2 (5) and expressed in terms of monomer ($M_0 = 25,000$).

MeOD, MeOH, Man1–2-ManOAc, Man1–3-ManOAc, Man1–6-ManOAc, and 1 were purchased from Sigma. MeOD2Man was a gift from Dr. S. Sabesan. Synthesis of deoxy analogs 2–11 in Fig. 1 has been reported (6). Concentration of carbohydrates was determined by modification of the Dubois phenol-sulfuric acid method (7) using appropriate monosaccharides (Man, 2-deoxymannose, 3-deoxymannose, 4-deoxymannose, and 6-deoxymannose) as standards. Deuterium oxide (99.9% deuterium) was obtained from Sigma.

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Thermodynamic Binding Studies—ITC was performed using an MCS microcalorimeter from Microcal, Inc. (Northampton, MA). In individual titrations, injections of 4 μl of carbohydrate were added from the computer-controlled 100-μl microsyringe at an interval of 4 min into the lectin solution (cell volume = 1.35 ml) dissolved in the same buffer as the saccharide, while stirring at 350 rpm. Control experiments performed by making identical injections of saccharide into a cell containing buffer with no protein showed insignificant heats of dilution. The experimental data were fitted to a theoretical titration curve using software supplied by Microcal, with $\Delta H$ (enthalpy change in kcal mol$^{-1}$), $K_a$ (association constant in M$^{-1}$), and $n$ (number of binding sites per monomer), as adjustable parameters. The quantity $c = K_a M_0(0)$, where $M_0(0)$ is the initial macromolecule concentration, is of importance in titration microcalorimetry (8). All experiments were performed with $c$ values $1 < c < 200$. The instrument was calibrated using the calibration kit containing RNase A and 2'-CMP supplied by the manufacturer. Thermodynamic parameters were calculated from the equation,

$$\Delta G = \Delta H - T \Delta S = -RT \ln K_a \quad (\text{Eq. 1})$$

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 mol$^{-1}$ K$^{-1}$.

Experiments performed in deuterium oxide were found to have >99% deuterium by $^1H$ nuclear magnetic resonance spectrometer analysis of the solutions. The buffer used in H$_2$O and D$_2$O was 0.1 M Hepes, 1 M NaCl, pH 7.2 (adjusted for the 0.4 pH difference in D$_2$O). The time that the two proteins were in the D$_2$O solvent over a week did not alter the results.

RESULTS AND DISCUSSION

ITC Data for DGL and ConA Binding to Trimannoside 1 in H$_2$O and D$_2$O—The results of ITC measurements of DGL and ConA binding to trimannoside 1 in H$_2$O and D$_2$O at pH 7.2 and 27 °C are shown in Tables I and II, respectively. The data show that both lectins exhibit significant primary solvent isotope effects in their $\Delta H$ values. The solvent isotope effects on $\Delta H$ for both lectins binding to 1 are proportional to the percentage of deuterium in the solvent as shown in Fig. 2 for DGL. In greater than 99% D$_2$O, the difference in $\Delta H$ values for DGL in H$_2$O and D$_2$O (Δ$\Delta H$ H$_2$O – D$_2$O) is 2.9 kcal mol$^{-1}$. The $\Delta H$ (H$_2$O) value for ConA is 2.0 kcal mol$^{-1}$. Thus, the solvent isotope effect in $\Delta H$ for DGL binding to 1 is greater than that for ConA.

The ITC data in Tables I and II also show that the $\Delta G$ values for the two lectins binding to 1 do not significantly change in H$_2$O and D$_2$O. $\Delta G$ for ConA in H$_2$O and D$_2$O is ~7.6 kcal mol$^{-1}$ and ~7.5 kcal mol$^{-1}$, respectively. $\Delta G$ for DGL in H$_2$O and D$_2$O is ~8.3 kcal mol$^{-1}$ and ~8.1 kcal mol$^{-1}$, respectively. This indicates that enthalpy-entropy compensation occurs for binding of the two lectins to 1 in the two solvents. This will be further discussed below.

Chervenak and Toone (9) reported much smaller solvent isotope effects on $\Delta H$ for ConA and DGL binding to 1 in H$_2$O and D$_2$O. $\Delta \Delta H$ (H$_2$O – D$_2$O) was 0.5 kcal mol$^{-1}$ for ConA and 0.4 kcal mol$^{-1}$ for DGL. Moreover, Chervenak and Toone also report $\Delta H$ values of ~13.0 kcal mol$^{-1}$ and ~10.7 kcal mol$^{-1}$ for DGL and ConA binding to 1, respectively, which are much lower than those reported here and in our previous ITC studies of ConA (~16.2 kcal mol$^{-1}$ for DGL and ~14.7 kcal mol$^{-1}$ for ConA) (1, 10, 11). We have consistently obtained the values in Table I using two different ITC instruments, the Omega and MCS instruments from Microcal Inc.

Since the primary hydrogen bonding contacts for DGL and ConA with trimannoside 1 are essentially the same (16), differences in the $\Delta \Delta H$ (H$_2$O – D$_2$O) values for the two lectins suggest that other factors are involved. One of these could be differences in the ordered water molecules in the extended binding site regions of the ConA and DGL- trimannoside complexes observed in their respective x-ray crystal structures (2) (16). In order to investigate this possibility, ITC primary solvent isotope studies in H$_2$O and D$_2$O were carried out with the two lectins and deoxy analogs 2–11 in Fig. 1. Primary solvent isotope effects have been used to characterize the kinetic and thermodynamic involvement of hydrogen bonding between water and donor/acceptor groups in small molecules and in protein complexes (cf. Refs. 12 and 13).

ITC Data for DGL and ConA Binding to Deoxy Trimannoside Analogs 2–11 in H$_2$O and D$_2$O—Tables I and II show the results of ITC measurements of the binding of DGL and ConA, respectively, to deoxy analogs 2–11 (Fig. 1) in H$_2$O and D$_2$O. To
aid in the comparison of data, the $\Delta H$ ($H_2O - D_2O$) results for the two lectins are shown in Fig. 3. Of the deoxy analogs of the $\alpha(1-3)$ arm of 1, the 2-deoxy analog (2) shows a $\Delta H$ ($H_2O - D_2O$) value of 3.3 kcal mol$^{-1}$ for DGL binding compared with a value of 1.2 kcal mol$^{-1}$ for ConA. The other three deoxy analogs of the $\alpha(1-3)$ arm, 3–5, (Fig. 1) showed far smaller differences in their $\Delta H$ ($H_2O - D_2O$) values (1.3–1.8 kcal mol$^{-1}$).

The $\Delta H$ ($H_2O - D_2O$) data for binding of DGL and ConA to deoxy analogs of the $\alpha(1-6)$ arm of 1 are shown in Tables I and II, respectively, as compared with ConA. The DGL value is 2.3 kcal mol$^{-1}$ as compared with 0.3 kcal mol$^{-1}$ for ConA. These values contrast with relatively similar values for DGL (1.9 kcal mol$^{-1}$) and ConA (1.5 kcal mol$^{-1}$) binding the 2-deoxy analog 10.

**Table I**

| Sugar       | Solvent | $K_a^{a}$ | $-\Delta H^{b}$ | $\Delta H^c (H_2O - D_2O)$ | $-\Delta E^d$ | $-\Delta G$ |
|-------------|---------|-----------|------------------|-----------------------------|----------------|-------------|
|             |         | $\mu^{-1} \times 10^{-3}$ | kcal/mol          | kcal/mol                    | kcal/mol       | kcal/mol     |
| MecMan$^{d}$ | $H_2O$  | 0.6       | 8.4              | 1.7                         | 3.3            | 5.1         |
| MecGlc$^{d}$ | $H_2O$  | 0.14      | 5.1              | 0.8                         | 0.8            | 4.3         |
| Mec2dMan$^{d}$ | $H_2O$ | 0.25      | 7.6              | 1.4                         | 3.0            | 4.6         |
| Maltose     | $H_2O$  | 0.29      | 6.1              | 0.7                         | 1.4            | 4.7         |
| Isomaltose  | $H_2O$  | 0.27      | 6.3              | 1.1                         | 1.6            | 4.7         |
| Man$\alpha(1-2)$Man$^{d}$ | $H_2O$ | 3.0       | 9.9              | 1.3                         | 3.8            | 6.1         |
| Man$\alpha(1-3)$Man$^{d}$ | $H_2O$ | 1.8       | 10.1             | 1.4                         | 4.3            | 5.8         |
| Man$\alpha(1-6)$Man$^{d}$ | $H_2O$ | 0.56      | 8.6              | 1.1                         | 3.5            | 5.1         |
| 1, trimannoside$^{d}$ | $H_2O$ | 121       | 16.2             | 2.9                         | 7.9            | 8.3         |
| 2, of 1-32-deoxy$^{d}$ | $H_2O$ | 70        | 15.2             | 3.3                         | 7.1            | 8.1         |
| 3, of 1-33-deoxy$^{d}$ | $H_2O$ | 4.8       | 11.2             | 1.3                         | 4.8            | 6.4         |
| 4, of 1-34-deoxy$^{d}$ | $H_2O$ | 56        | 14.6             | 1.2                         | 6.7            | 7.9         |
| 5, of 1-36-deoxy$^{d}$ | $H_2O$ | 74        | 15.1             | 1.8                         | 7.1            | 8.0         |
| 6, of 1-62-deoxy$^{d}$ | $H_2O$ | 1.2       | 15.0             | 1.6                         | 5.5            | 7.3         |
| 7, of 1-62-deoxy$^{d}$ | $H_2O$ | 2.6       | 13.0             | 1.2                         | 4.0            | 6.0         |
| 8, of 1-64-deoxy$^{d}$ | $H_2O$ | 2.4       | 10.4             | 1.8                         | 4.4            | 6.0         |
| 9, of 1-66-deoxy$^{d}$ | $H_2O$ | 1.9       | 9.8              | 1.2                         | 4.0            | 5.8         |
| 10, "core"2-deoxy$^{d}$ | $H_2O$ | 25        | 14.8             | 1.9                         | 7.4            | 7.3         |
| 11, "core"4-deoxy$^{d}$ | $H_2O$ | 11        | 12.8             | 2.3                         | 5.9            | 6.9         |

$^{a}$ Errors in $K_a$ values are between 2 and 10%.
$^{b}$ Errors in $\Delta H$ and $-\Delta E$ are $\pm 0.1 - 0.2$ kcal mol$^{-1}$.
$^{c}$ Errors in $\Delta H$ are $\pm 0.2 - 0.4$ kcal mol$^{-1}$.
$^{d}$ Data taken from Ref. 15.
$^{e}$ Man$\alpha(1-2)$Man is Man$\alpha(1-2)$ManOMe.
$^{f}$ Man$\alpha(1-3)$Man is Man$\alpha(1-3)$ManOMe.
$^{g}$ Man$\alpha(1-6)$Man is Man$\alpha(1-6)$ManOMe.
The buffer was 0.1 M Hepes containing 0.9 M NaCl, 1 mM Mn^{2+}, and 1 mM Ca^{2+} at pH 7.2 in H_2O and D_2O. Values of n were between 0.98 and 1.03 in all cases.

**TABLE II**

| Sugar          | Solvent | K_a | \(\Delta H^b\) | \(\Delta S^b\) | \(\Delta G^b\) |
|----------------|---------|-----|----------------|----------------|----------------|
|                |         | \(\mu^{-1} \times 10^{-4}\) | kcal/mol | kcal/mol | kcal/mol |
| MecMan^c       | H_2O    | 1.2 | 8.4           | 0.5            | 2.8            | 5.6            |
| MecGlc         | H_2O    | 0.23| 6.7           | 0.4            | 2.1            | 4.6            |
| Mee2dMan       | H_2O    | 0.67| 7.2           | 0.7            | 1.9            | 5.2            |
| Maltose        | H_2O    | 0.13| 6.2           | 0.3            | 1.9            | 4.3            |
| Isomaltose     | H_2O    | 0.51| 6.7           | 0.4            | 1.6            | 5.1            |
| Manα(1→2)Manβ | H_2O    | 11  | 10.6          | 0.5            | 3.8            | 6.8            |
| Manα(1→3)Manβ | H_2O    | 3.3 | 10.7          | 0.9            | 4.5            | 6.2            |
| Manα(1→6)Manβ | H_2O    | 0.81| 8.4           | 0.8            | 3.1            | 5.3            |
| 1, trimannoside | H_2O | 39  | 14.7          | 2.0            | 7.1            | 7.6            |
| 2, of 1→3-deoxy| H_2O | 19  | 14.1          | 1.2            | 6.9            | 7.2            |
| 3, of 1→35-deoxy| H_2O | 5.4 | 11.0          | 0.8            | 4.5            | 6.5            |
| 4, of 1→34-deoxy| H_2O | 9.2 | 12.3          | 0.7            | 5.5            | 6.8            |
| 5, of 1→36-deoxy| H_2O | 39  | 14.0          | 1.2            | 6.4            | 7.7            |
| 6, of 1→6-deoxy| H_2O | 16  | 14.0          | 0.7            | 6.9            | 7.1            |
| 7, of 1→6-deoxy| H_2O | 2.8 | 11.2          | 1.4            | 4.9            | 6.3            |
| 8, of 1→64-deoxy| H_2O | 2.5 | 11.7          | 1.5            | 5.7            | 6.0            |
| 9, of 1→6-deoxy| H_2O | 3.0 | 11.6          | 1.3            | 5.5            | 6.1            |
| 10, "core"2-deoxy| H_2O | 12  | 13.4          | 1.5            | 6.5            | 6.9            |
| 11, "core"4-deoxy| H_2O | 4.6 | 12.1          | 0.3            | 5.7            | 6.4            |

| Sugar          | Solvent | K_a | \(\Delta H^b\) | \(\Delta S^b\) | \(\Delta G^b\) |
|----------------|---------|-----|----------------|----------------|----------------|
| MecMan^c       | D_2O    | 1.1 | 7.9           | 2.4            | 5.5            |
| MecGlc         | D_2O    | 0.31| 6.3           | 1.6            | 4.7            |
| Mee2dMan       | D_2O    | 0.37| 6.5           | 1.6            | 4.9            |
| Maltose        | D_2O    | 0.14| 5.9           | 1.6            | 4.3            |
| Isomaltose     | D_2O    | 0.53| 6.3           | 1.2            | 5.1            |
| Manα(1→2)Manβ | D_2O    | 9.2 | 10.1          | 3.2            | 6.7            |
| Manα(1→3)Manβ | D_2O    | 3.6 | 9.8           | 3.6            | 6.2            |
| Manα(1→6)Manβ | D_2O    | 0.76| 7.6           | 2.3            | 5.3            |
| 1, trimannoside | D_2O | 34  | 12.7          | 5.2            | 7.5            |
| 2, of 1→32-deoxy| D_2O | 18  | 12.9          | 5.7            | 7.2            |
| 3, of 1→33-deoxy| D_2O | 5.4 | 10.2          | 3.8            | 6.4            |
| 4, of 1→34-deoxy| D_2O | 20  | 11.6          | 4.4            | 7.2            |
| 5, of 1→36-deoxy| D_2O | 30  | 12.9          | 5.3            | 7.5            |
| 6, of 1→62-deoxy| D_2O | 15  | 13.3          | 6.2            | 7.1            |
| 7, of 1→63-deoxy| D_2O | 4.3 | 9.8           | 3.5            | 6.3            |
| 8, of 1→64-deoxy| D_2O | 4.0 | 10.2          | 3.9            | 6.3            |
| 9, of 1→66-deoxy| D_2O | 4.7 | 10.3          | 3.9            | 6.4            |
| 10, "core"2-deoxy| D_2O | 19  | 11.9          | 4.7            | 7.2            |
| 11, "core"4-deoxy| D_2O | 7.5 | 11.8          | 5.2            | 6.6            |

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

\(b\) Errors in \(\Delta H\) and \(\Delta S\) are \(\pm 0.1–0.2\) kcal mol\(^{-1}\).

\(c\) Errors in \(\Delta \Delta H\) are \(\pm 0.2–0.4\) kcal mol\(^{-1}\).

\(d\) Data taken from Ref. 15.

\(e\) Manα(1→3)Man is Manα(1→2)ManαMe.

\(f\) Manα(1→3)Man is Manα(1→3)ManαMe.

\(g\) Manα(1→6)Man is Manα(1→6)ManαMe.

\(h\) Data taken from Mandal et al. (11) and included here for comparison.

\(i\) Data taken from Gupta et al. (1) and included here for comparison.

...Thus, little difference is observed in these \(\Delta \Delta H\) (H_2O – D_2O) for DGL and ConA.

Manα(1→2)ManαMe, Manα(1→3)ManαMe, and Manα(1→4)ManαMe were also tested, and the results are shown in Tables I and II. The \(\Delta \Delta H\) (H_2O – D_2O) values for DGL binding were 1.3, 1.4, and 1.1 kcal mol\(^{-1}\), respectively. The \(\Delta \Delta H\) (H_2O – D_2O) values for ConA were 0.5, 0.9, and 0.8 kcal mol\(^{-1}\), respectively. Thus, there is a slightly larger difference in the Manα(1→2)ManαMe values for the two lectins as compared with the differences observed for the other two Man disaccharides.

Correlation of the \(\Delta \Delta H\) (H_2O – D_2O) Data for Analogues 2–11 with Differences in the Location of Ordered Water in the DGL and ConA Complexes with Trimannoside 1—Fig. 4 shows schematic representations of the x-ray crystal structures of the binding site regions of ConA and DGL complexed to the core trimannoside. The structures show the presence of ordered water in the two complexes (16). Although the primary contacts between the two lectins and the trimannoside are essentially the same in the two complexes, the location of ordered water molecules in the binding site regions differs in the complexes. This is due to amino acid substitutions in the regions adjacent to the contact residues in both proteins.

The x-ray data reveal differences in the location of ordered water in the following three regions of the two complexes. The first region is associated with the 2-hydroxyl group on the α(1→3) Man of 1. In the ConA complex (Fig. 4), the 2-hydroxyl on the α(1→3) Man interacts with W69 and indirectly with W58 via W69. In the DGL complex (Fig. 4), the 2-hydroxyl interacts with W69 and W68. W69, in turn, interacts with W70 and W68. W68 interacts with W59 and the carbonyl oxygen of Asn21. Thus, the location of ordered water around the 2-hydroxyl on the α(1→3) Man of 1 is different in the two complexes.

The \(\Delta \Delta H\) (H_2O – D_2O) data for the α(1→3) deoxy analogs of 1 show that only the 2-deoxy analog (2) exhibits a larger variation in the \(\Delta \Delta H\) (H_2O – D_2O) for DGL and ConA (Tables I and II; Fig. 3). \(\Delta \Delta H\) (H_2O – D_2O) for DGL is 3.3 kcal mol\(^{-1}\) as compared with 0.8 kcal mol\(^{-1}\) for ConA. The other three analogs, 3–5, show much smaller differences in their \(\Delta \Delta H\) (H_2O – D_2O) values.
are both present in the two complexes, with both binding to the 2-hydroxyl on the α(1–6) arm. W87, however, is adjacent to a water molecule bonded to Ser168 in ConA, while in DGL W87 is adjacent to the side chain carbonyl oxygen of Asn168. In addition, W67, which is adjacent to W66 in both complexes, is bonded to the carbonyl oxygen of Thr226 in ConA but to the carbonyl oxygen of Gly226 in DGL. Furthermore, W66 in ConA is directly bonded to the side chain hydroxyl of Thr226, but in DGL W66 is bonded to W89. Thus, the secondary ordered water layer near the 2-hydroxyl on the α(1–6) arm of 1 is different in the two complexes.

The ΔΔH (H2O – D2O) data for DGL and ConA binding to the deoxy analogs of the α(1–6) arm of 1 show that the 2-deoxy derivative (2) possesses the largest difference in their respective values. The ΔΔH (H2O – D2O) value for DGL is 1.6 kcal mol⁻¹, while the ΔΔH (H2O – D2O) value for ConA is 0.7 kcal mol⁻¹. The other three analogs, 3–5, show much smaller differences (Tables I and II; Fig. 3).

Correlation of ΔΔH (H2O – D2O) Values of Deoxy Analogs of 1 with the Number and Strength of Solvent Hydrogen Bonds to Hydroyl Groups of Trimmansoside 1 in DGL and ConA—The relative magnitude of the ΔΔH (H2O – D2O) values of DGL and ConA in Tables I and II for deoxy analogs 2, 6, and 11 also show a correlation with the numbers and strength of water molecules interacting with corresponding hydroxyl groups of the parent trimannoside in the respective complexes. For example, the largest ΔΔH (H2O – D2O) value for DGL is for binding 2 (3.3 kcal mol⁻¹). This contrasts with the corresponding ConA value of 1.2 kcal mol⁻¹. Fig. 4 shows that in DGL the 2-hydroxyl of the α(1–3) Man of 1 is directly hydrogen-bonded to two water molecules (W69 and W68), while in ConA only one water molecule (W69) is observed to bind to this hydroxyl group. Furthermore, W69 is closer to the hydroxyl group in DGL (3.1 Å) than in ConA (3.4 Å). Thus, the number and apparent strength of hydrogen-bonding water molecules to the 2-hydroxyl of the α(1–3) Man of 1 appears greater in DGL than in ConA.

The next largest value of ΔΔH (H2O – D2O) for DGL is 2.3 kcal mol⁻¹ for 11. The corresponding value for ConA is 0.3 kcal mol⁻¹. Fig. 4 shows that the 4-hydroxyl of the core Man of 1 is directly bonded to W41 in DGL. However, there is an absence of electron density for a solvent molecule at this position in the ConA complex.

Thus, differences in numbers and strength of the water molecules in DGL and ConA interacting with specific hydroxyl groups of trimannoside 1 appear to be reflected in the magnitude of the ΔΔH (H2O – D2O) values for 2, 6, and 11.

Correlation of the ΔΔH (H2O – D2O) Data for MeoMan and MeoGlc with Differences in the Location of Ordered Water in the DGL and ConA Complexes with Trimmansoside 1—The ΔΔH (H2O – D2O) values for MeoMan and MeoGlc in Tables I and II also show a correlation with the altered ordered water structures observed in the binding site regions of the DGL and ConA complexes with the trimannoside (Fig. 4). Since MeoMan occupies the same site as the α(1–6) Man residue of 1 and makes similar contacts with ConA (2), it is reasonable to assume that the altered ordered water near the 2-hydroxyl of the α(1–6) Man residue of 1 in the DGL and ConA complexes is present in their respective complexes with the monosaccharide. The ΔΔH (H2O – D2O) value for DGL binding to MeoMan (1.7 kcal mol⁻¹) is considerably greater than that for ConA (0.5 kcal
mol$^{-1}$), which is consistent with altered solvation of these two lectin complexes. Furthermore, the $\Delta \Delta H$ (H$_2$O $-$ D$_2$O) values for DGL binding to Me$_2$Man (1.7 kcal mol$^{-1}$) and MeoGlc (0.8 kcal mol$^{-1}$) are substantially different, while the corresponding values for ConA binding to the two monosaccharides of 0.5 and 0.4 kcal mol$^{-1}$, respectively, are not. Since the two sugars differ in the orientation of their 2-hydroxyl groups (axial and equatorial, respectively), these results are consistent with altered solvation of the two monosaccharide complexes in both lectins, specifically at the 2-axial hydroxyl group of Man in both lectins.

The $\Delta \Delta H$ (H$_2$O $-$ D$_2$O) data for DGL and ConA binding to Me$_2$doMan in Tables I and II are also suggestive of differential solvation of the respective complexes. The $\Delta \Delta H$ (H$_2$O $-$ D$_2$O) data for DGL binding to the monosaccharide is 1.4 kcal mol$^{-1}$, while for ConA the value is 0.7 kcal mol$^{-1}$.

Interestingly, no similar obvious trend in the $\Delta \Delta H$ (H$_2$O $-$ D$_2$O) data is observed for the Man and Glc disaccharides listed in Tables I and II. This suggests that solvation of these longer oligosaccharides in the respective DGL and ConA complexes moderates any differential solvation effects observed for the monosaccharides, trimannoside 1 and its deoxy analogs.

Lack of Correlation of Altered Water Structures in the DGL and ConA Complexes with the Core Trimannoside and $\Delta \Delta H$ Values in H$_2$O for Binding of Both Lectins to the Deoxy Analogs of Trimannoside 1—The altered water structure observed in Fig. 4 for the ConA and DGL complex complexes with trimannoside 1 is consistent with the observed $\Delta \Delta H$ (H$_2$O $-$ D$_2$O) values of deoxy analogs 2, 6, and 11. This provides confirmation that the differences in the ordered water structures observed in the x-ray crystal complexes of the two lectins with the core trimannoside exist in their corresponding solution complexes. These results allow consideration of the contribution of altered structural water near the $\alpha$(1–6) Man of 1 in the two complexes.

FIG. 4. Schematic representation of the hydrogen bond interactions between the trimannoside ligand and the surrounding amino acid residues and ordered water molecules in ConA and in DGL. The dashed lines represent hydrogen bonds and the distances are labeled in Å. Water 39 has strong electron density; is held by the side chains of Asn$^{14}$, Asp$^{16}$, and Arg$^{228}$; and makes a direct hydrogen bond with the trimannoside ligand; and its position is strictly conserved between DGL and ConA. The remaining ordered water molecules possess weaker electron density, which may indicate a decrease in binding strength between themselves and the protein.

FIG. 5. Plots of $\Delta \Delta H$ versus $-\Delta \Delta S$ for the binding of DGL to the carbohydrates in Table I in H$_2$O (■) and D$_2$O (●) (A) and ConA to the carbohydrates in Table II in H$_2$O (■) and D$_2$O (●) (B). The solid lines are fits of the data.
to the differences in the $\Delta H$ values of the deoxy analogs of the $\alpha(1-6)$ Man of 1 reported in the first paper in this series (15).

The $\Delta H$ values of DGL in H$_2$O for the 2-, 3-, 4-, and 6-deoxy analogs of 1 (6–9) in Fig. 1 are observed to be $-3$ kcal mol$^{-1}$ greater than the corresponding values in ConA (15). However, as shown in Fig. 4, and together with the $\Delta H$ (H$_2$O – D$_2$O) data in the present study, the altered water structure in that region of the two complexes appears to effect primarily the 2-hydroxyl of the $\alpha(1-6)$ Man of 1 and not the 3-, 4-, and 6-hydroxy groups. The 3-hydroxyl of the $\alpha(1-6)$ Man of 1 is in contact with W60 in both complexes, while the 4- and 6-hydroxy groups are not directly bonded to water molecules. In addition, the $\Delta H$ values of DGL and ConA in D$_2$O for deoxy analogs 7–9 show a difference of $-2.0$ kcal mol$^{-1}$ as compared with the $-3$ kcal mol$^{-1}$ difference in H$_2$O. On the other hand, the 2-deoxy analog (6) shows a reversal (0.8 kcal mol$^{-1}$) of this difference between the two lectins in H$_2$O and D$_2$O (Tables I and II). Thus, the altered water structure near the $\alpha(1-6)$ Man of 1 may account for the differences in the $\Delta H$ values of DGL and ConA for the 2-deoxy analog (2) to di- and trideoxy analogs of 1 (1). The slope of the data in D$_2$O is 1.59 with a correlation coefficient of 0.97. Thus, the solvent isotope effect on ConA binding to the carbohydrates in Fig. 1 is evident in the enthalpy-entropy compensation plots of the data in Table II.

Summary—The present study demonstrates that ITC measurements of solvent isotope effects in the $\Delta H$ values of DGL and ConA binding to certain monosaccharides and deoxy analogs of trimannoside 1 in H$_2$O and D$_2$O are sensitive to altered solvation of the core trimannoside at specific sites, consistent with the x-ray crystal structures of the two respective lectin complexes. Differences in the numbers and strength of the water molecules in DGL and ConA interacting with specific hydroxyl groups of trimannoside 1 appear to be reflected in the magnitude of the $\Delta H$ (H$_2$O – D$_2$O) values for 2, 6, and 11. Thus, evidence that solvation differences exist in solution for the DGL and ConA complexes with 1 has been obtained. The results allow us to conclude that differences in the $\Delta H$ values of $\alpha(1-6)$ deoxy analogs of 1 binding to DGL and ConA in H$_2$O do not correlate with solvation differences of the parent trimannoside complexes. The origin of these differences is presently being investigated.

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Differential Solvation of "Core" Trimannoside Complexes of the *Dioclea grandiflora* Lectin and Concanavalin A Detected by Primary Solvent Isotope Effects in Isothermal Titration Microcalorimetry

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*J. Biol. Chem.* 1998, **273**:32826-32832.
doi: 10.1074/jbc.273.49.32826

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