Isothermal Titration Calorimetric Studies on the Binding of Deoxytrimannoside Derivatives with Artocarpin: Implications for a Deep-Seated Combining Site in Lectins†

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ABSTRACT: The carbohydrate binding specificity of the seed lectin from *Artocarpus integrifolia*, artocarpin, has been elucidated by the enzyme-linked lectin absorbent assay [Misquith, S., et al (1994) *J. Biol. Chem. 269*, 30393−30401], wherein it was demonstrated to be a Man/Glc specific lectin with high affinity for the trisaccharide present in the core of all N-linked oligosaccharide chains of glycoproteins. As a consequence of this characterization, the binding epitopes of this trisaccharide, 3,6-di(α-D-mannopyranosyl)-D-mannose, for artocarpin were investigated by isothermal titration calorimetry using its monodeoxy as well as Glc and Gal analogues. The thermodynamic data presented here implicate 2-, 3-, 4-, and 6-hydroxyl groups of the α(1−3) Man and α(1−6) Man residues, and the 2- and 4-OH groups of the central Man residue, in binding to artocarpin. Nevertheless, α(1−3) Man is the primary contributor to the binding affinity, unlike other Man/Glc binding lectins which exhibit a preference for α(1−6) Man. In addition, unlike the binding reactions of most lectins reported so far, the interaction of mannotriose involves all of its hydroxyl groups with the combining site of the lectin. Moreover, the free energy and enthalpy contributions to binding of individual hydroxyl groups of the trimannoside estimated from the corresponding monodeoxy analogues show nonlinearity, suggesting differential contributions of the solvent and protein to the thermodynamics of binding of the analogues. Thus, this study not only provides evidence for the extended site recognition of artocarpin for the trimannoside epitope but also suggests that its combining site is best described as a deep cleft as opposed to shallow indentations implicated in other lectins.

Lectins, proteins of nonimmune origin, bind to sugars reversibly and with a very high degree of specificity (*1*, *2*). The recognition of carbohydrate moieties by lectins has several implications in a number of biological processes such as cellular adhesion (*3*), cellular recognition (*4*), and signal transduction (*5*, *6*), and can in addition affect cell growth and differentiation (*2*, *7*). The ability of plant lectins to detect subtle variations in carbohydrate structures found on cell surface glycoproteins and glycolipids has made them a paradigm for protein−carbohydrate recognition (*8*, *9*). Sugar binding proteins, e.g., enzymes that act on sugars as substrates, carbohydrate specific antibodies, membrane transport proteins, and bacterial periplasmic proteins involved in sugar mobilization and chemotaxis form the group I family of carbohydrate binding proteins (*10*, *11*). Here the sugar binding sites are located in deep clefts, and the binding reaction leaves hardly any portion of the ligand exposed to the bulk solvent. Lectins, on the contrary, belong to the group II family of carbohydrate binding proteins which have shallow depressions on their surface as sugar binding sites (*12*−*14*).

Artocarpin, a nonglycosylated tetrameric protein with an M_r of 64 000 Da, belongs to the class of mannose specific plant lectins, notably, ConA, pea, lentil, and snowdrop lectin (*15*). Artocarpin has recently received considerable attention because of its ability to trigger the maturation of B lymphocytes in vitro and also because of its unique carbohydrate specificity (*16*, *17*). Our earlier carbohydrate specificity studies using the enzyme-linked lectin absorbent assay (ELLA) have shown that the mannose/glucose specific lectin from the seeds of *Artocarpus integrifolia* (Jack fruit) binds with high affinity to the "core" trisaccharide, 3,6-di(α-D-mannopyranosyl)-D-mannose which is present in asparagine-linked carbohydrates (*15*). Detailed insight into the specificity of carbohydrate−protein interactions requires not only information about relative binding affinity but also thermodynamic data to suggest if any extended binding site interactions occur. Calorimetric techniques have played an important role in the acquisition of thermodynamic data to

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aid in the design and synthesis of inhibitors of carbohydrate-recognizing proteins. Moreover, it has provided much stimulus to the development of algorithms for predicting protein−ligand interaction energetics which are necessary for the structure-based design of therapeutic agents (*18*). And indeed, the isothermal titration calorimetry data show that in artocarpin the α(1−3)-linked mannose occupies the primary binding site, and the α(1−6) Man occupies the secondary subsite with both sites being specific to the α-(1−3) and α(1−6) arms of the two oligosaccharides. They, together with the 3,6-disubstituted mannose residue, constitute the extended combining site of artocarpin (*19*). Moreover, such studies conducted with the deoxy analogues of the sugar also allow one to dissect the manner in which hydroxyl groups participate in the binding reaction (*20*, *21*). This is borne out very succinctly using a series of systematically modified deoxy analogues of the parent compound which enables one to unambiguously define the involvement of the various hydroxyl groups to the overall binding thermodynamics for a given reaction. A comparison of the change in the thermodynamic parameters, namely, $\Delta H_b°$, $\Delta G_b°$, and $\Delta S_b°$, between the reference analogues and the parent compound helps to highlight the role of individual hydroxyl groups in the binding energetics. The study presented here deals with the thermodynamic analyses of the binding of a series of Man 3,6 derivatives possessing either glucose or galactose substituted on either of the arms of the trisaccharide, and a complete set of monodeoxy derivatives of the α(1−3) arm, the α(1−6) arm, and the core Man residue of the trimannoside. Interestingly, unlike any other lectin studied so far, artocarpin appears to recognize all the hydroxyl groups of its complementary ligand. Consequently, its combining site can be considered to be a deep cleft, in contrast to the lectins studied so far where it is essentially a shallow depression at the surface of the protein. This study gains significance as it adds to the existing knowledge of the mode of recognition and interaction of lectins with cellular carbohydrate receptors.

EXPERIMENTAL PROCEDURES

Materials and Sample Preparation. Synthesis of glucose, galactose, and monodeoxy trimannoside derivatives was as reported earlier by Oscarson and co-workers (*22*, *23*). All other reagents and chemicals were of the highest purity available. Artocarpin, as purified according to Misquith et al. (*15*), was dialyzed overnight against a large volume (3000×) of 20 mM sodium phosphate buffer (pH 7.4) containing 150 mM sodium chloride (PBS) and centrifuged to remove any insoluble material. The concentration of the protein was determined spectrophotometrically. The ligand solutions were prepared by weight in the final dialysate to minimize differences between the protein buffer solution and the ligand buffer solution in the ITC measurements.

Titration Calorimetry. Isothermal titration calorimetric measurements were performed using an OMEGA titration calorimeter from Microcal Inc., as described previously by Wiseman et al. (*24*) and Surolia et al. (*25*). A circulating water bath was employed to help temperature stabilization. The instrument was allowed to equilibrate overnight. Aliquots (5−10 μL) of the ligand solution were added from the computer-controlled 250 μL rotating syringe; the contents were stirred at 395 rpm into the cell containing 1.385 mL of the lectin solution with 3 min between each injection allowing the baseline to stabilize. The total concentration of the lectin M_t was varied from 0.5 to 2 mM, whereas the total concentration of the ligand X_t taken for the titration was varied from 8 to 64 mM. The titration of the ligand solution in this concentration range with the buffer solution alone gave negligible values for the heats of dilution. The unitless parameter C [$=K_bM_t(0)$, where K_b is the binding constant and $M_t(0)$ the initial macromolecular concentration] is important in titration calorimetry experiments. All experiments described in this study were performed with C values between 1 and 200.

The total heat Q_t was then fitted via a nonlinear least-squares minimization method to the total ligand concentration (X_t) using the following equation.

$$Q_t = nM_t\Delta H_b V\{1 + X_t/nM_t + 1/nK_bM_t - [(1 + X_t/nM_t + 1/nK_bM_t)^2 - 4X_t/nM_t]^{1/2}\}/2 \quad (1)$$

where n is the number of binding sites per monomer and V is the cell volume. The expression for the heat released for the ith injection, $\Delta Q(i)$, is then given by eq 2 (*26*)

$$\Delta Q(1) = \Delta Q(i) + dV_i/2V[Q(i) + Q(i-1)] - Q(1-1) \quad (2)$$

where dV_i is the volume of titrant added to the solution

$$\Delta G_b° = \Delta H_b° - T\Delta S_b° \quad (3)$$

$$\Delta G_b° = -nRT \ln(K_b) \quad (4)$$

where n is the number of moles and T is the absolute temperature.

RESULTS

A typical titration calorimetry curve for the binding of Glc6 to artocarpin together with the incremental heats of interaction versus the injection number is shown in Figure 2. Because the incremental heats of binding per mole of added ligand closely follow the injection number as defined by eq 2 (as shown in Experimental Procedures), the lectin appears to have only one type of site for each saccharide. Thus, each ligand binds to the same site on the protein molecule. This is also proven by similar stoichiometries (1 ± 0.07) for the interaction of these ligands with the artocarpin monomer (M_r = 16 000). The values of the thermodynamic parameters thus obtained are listed in Table 1.

DISCUSSION

The thermodynamic parameters listed in Table 1 show that the replacement of mannose in either the 1−3 or 1−6 arm with either glucose or galactose leads to significant but variable losses in affinities and enthalpies. Additionally, a replacement of any of the hydroxyl groups of the trimannoside with a deoxy group is strikingly deleterious for the interaction of such ligands with artocarpin. Also, 6deoxy3 is completely inactive. Observation of compensatory changes in enthalpies with entropies for the binding of all these ligands is also consistent with the interpretation that all of them are recognized by artocarpin in a similar manner as well as the involvement of water molecules in mediating the

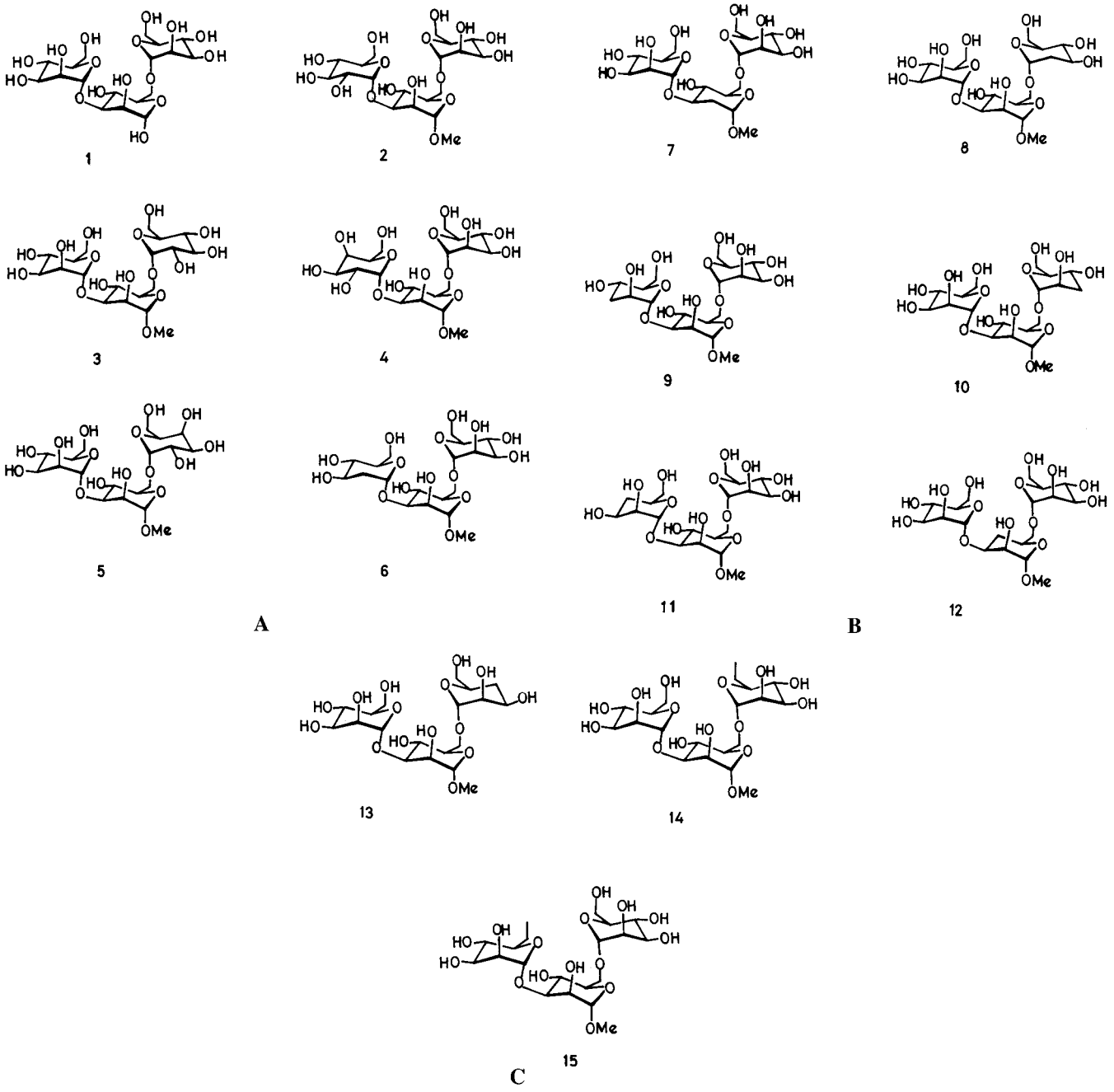

FIGURE 1: Structures of the trimannoside and its derivatives. (A) Trimannoside, 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannose (**1**); Glc3, methyl 3-*O*-(α-D-glucopyranosyl)-6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**2**); Glc6, methyl 3-*O*-(α-D-mannopyranosyl)-6-*O*-(α-D-glucopyranosyl)-α-D-mannopyranoside (**3**); Gal3, methyl 3-*O*-(α-D-galactopyranosyl)-6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**4**); Gal6, methyl 3-*O*-(α-D-mannopyranosyl)-6-*O*-(α-D-galactopyranosyl)-α-D-mannopyranoside (**5**); and 2deoxy3, methyl 6-*O*-(α-D-mannopyranosyl)-3-*O*-(2-deoxy-α-d-mannopyranosyl)-α-d-mannopyranoside (**6**). (B) 2Deoxyc, methyl 6-*O*-(α-D-mannopyranosyl)-3-*O*-(α-D-mannopyranosyl)-2-deoxy-α-D-mannopyranoside (**7**); 2deoxy6, methyl 6-*O*-(2-deoxy-α-D-mannopyranosyl)-3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**8**); 3deoxy3, methyl 6-*O*-(α-D-mannopyranosyl)-3-*O*-(3-deoxy-α-D-mannopyranosyl)-α-D-mannopyranoside (**9**); 3deoxy6, methyl 6-*O*-(3-deoxy-α-D-mannopyranosyl)-3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**10**); 4deoxy3, methyl 6-*O*-(α-D-mannopyranosyl)-3-*O*-(α-4-deoxy-α-D-mannopyranosyl)-α-D-mannopyranoside (**11**); and 4deoxyc, methyl 6-*O*-(α-D-mannopyranosyl)-3-*O*-(α-D-mannopyranosyl)-4-deoxy-α-d-mannopyranoside (**12**). (C) 4Deoxy6, methyl 6-*O*-(4-deoxy-α-D-mannopyranosyl)-3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**13**); 6deoxy6, methyl 6-*O*-(6-deoxy-α-D-mannopyranosyl)-3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**14**); and 6deoxy3, methyl 6-*O*-(α-D-mannopyranosyl)-3-*O*-(6-deoxy-α-D-mannopyranosyl)-α-D-mannopyranoside (**15**).

interaction between some of the hydroxyl groups of the saccharide and the corresponding loci in the combining site of the protein (Table 1). Also, the nonlinearity of the thermodynamic parameters described below is in agreement with such an observation. The thermodynamic data indicate that the $\Delta\Delta H$ values for the interaction of deoxy analogues or trimannoside with artocarpin exhibit nonlinearity (Table 2). For example, the sum of the $\Delta\Delta H$ values for monodeoxy analogues 2deoxy3 (**6**), 3deoxy3 (**9**), 4deoxy3 (**11**), and 6deoxy3 (**15**) is 105.3 kJ. To the first approximation, this represents the combined ΔH contribution of the 2-, 3-, 4-, and 6-hydroxyl groups of the α(1−3) Man residue which is greater than that of the parent trimannoside, indicating other contributions to these terms such as solvent and protein effects. Similarly, the sum of the $\Delta\Delta H$ values for the 2-, 3-, 4-, and 6-hydroxyl groups of α(1−6) Man [2deoxy6 (**8**),

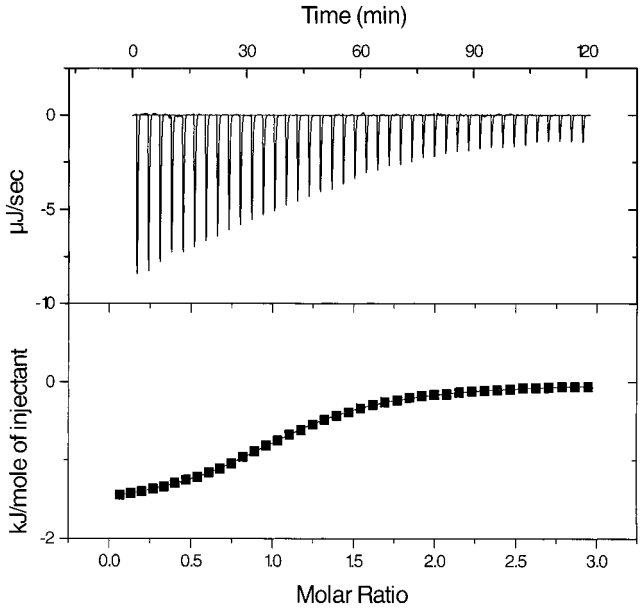

FIGURE 2: Calorimetric titration of artocarpin with Glc6 at 281 K. (Top) Data obtained for 50 automatic injections, with each injection being 6 μL of Glc6 (10 mM) in 1 mM artocarpin solution. (Bottom) The integrated curve shows experimental points (■) and the best fit (—). The buffer was 20 mM phosphate buffer (pH 7.4) containing 150 mM NaCl.

3deoxy6 (**10**), 4deoxy6 (**13**), and 6deoxy6 (**14**)] is 121.7 kJ. Furthermore, the sum of the $\Delta\Delta H$ values for the 2-, 3-, 4-, and 6-hydroxyl group of the α(1−3) Man (**6**, **9**, **11**, and **15**), the 2-, 3-, 4-, and 6-OH groups of the α(1−6) Man (**8**, **10**, **13**, and **14**), and the 2- and 4-OH groups of the central Man residue (**7** and **12**) is 295.4 kJ mol^{-1}, which is almost 7 times greater than the ΔH of **1** which is −44.80 kJ mol^{-1}. Thus, the sum of the $\Delta\Delta H$ values for the hydroxyl groups of **1** obtained from the monodeoxy analogue (Table 2) do not correspond to the measured ΔH of **1**. Besides, the sum of the $\Delta\Delta H$ values for specific hydroxyl groups on certain Man residues of **1** obtained from the corresponding monodeoxy analogues is greater than the measured ΔH for that residue(s). The same nonlinearity is also observed in the $\Delta\Delta G$ values of the monodeoxy analogues. Nonlinearity in the $\Delta\Delta H$ and $\Delta\Delta G$ values for the deoxy analogues in Table 2 represents differential contributions of the solvent and protein to the binding of **1** and its deoxy analogues to artocarpin and not just the loss of hydrogen bonds.

As the changes in binding enthalpies and entropies are compensatory in nature and as $\Delta S_b°$ is indirectly determined by a difference between two experimentally obtained parameters, i.e., $\Delta H_b°$ and $\Delta G_b°$ values, only the latter values for various ligands are compared (Table 1 and Figure 3). Though $\Delta H_b°$ and the relative binding affinities of various ligands are being compared at 281 ± 0.3 K, a similar correlation also holds true at the other temperature that was used (*25*, *27*−*29*). The analogues of the trimannoside containing either Glc or Gal with the substitution of the Man residue at either the α(1−3) or α(1−6) arm exhibit significantly reduced potencies as ligands for artocarpin. The substitution of glucose on the α(1−3) and α(1−6) arms results in about 18- and 3-fold loss, respectively, in the extent of binding and decreases in the values of ΔH of 36.6 and 37.8 kJ mol^{-1}, respectively, relative to that of the parent compound. As the replacement of the mannosyl residue with a glucosyl moiety at the α(1−3) arm causes a greater dimunition in activity than that observed for the replacement of the α(1−6) mannose, it is in order to conclude that the former occupies the primary site, viz., the monosaccharide binding site in the combining region of artocarpin. By the same token, the α(1−6) mannose residue of trimannoside occupies the secondary subsite. These data thus not only substantiate but also extend our earlier interpretations about the extended combining region in this lectin. Moreover, these data point out significant differences in the recognition of the same carbohydrate epitope by concanavalin A and a miscellany of lectins from the genus *Dioclea*, where the α-(1−6) mannose is complementary to their primary binding site (*30*).

The dimunition in the activities of Glc3 and Glc6 analogues relative to that of trimannoside could be a consequence of the loss of a favorable contact or the introduction of an unfavorable interface as the axially oriented C2 OH group of either the α(1−3) or α(1−6) mannose residue is replaced with an equatorially oriented hydroxyl group at C2 as in glucose. The fact that 2deoxy3 (**6**) is also a poor ligand implies that the axially oriented hydroxyl group of α(1−3)-linked mannose contributes to a favorable contact. Therefore, it is apparent that the poor binding of Glc3 analogue is due to a removal of such a favorable contact. The interaction at the C2 locus of the α-(1−3) mannose of **1** therefore differs significantly from that observed for ConA where an equatorially oriented hydroxyl group compromises the binding of Glc3 analogue in an unfavorable fashion (*30*).

Both Gal3 and Gal6 analogues are considerably weak as ligands; their affinities are decreased by 15- and 23-fold and their enthalpies compromised by 35.5 and 37.6 kJ mol^{-1}, respectively, when compared to those of the parent trimannoside. Since Gal3 is nearly as good a ligand as 4deoxy3 (**11**), it is obvious that the poor activity of the former can be explained on the basis of the loss of the favorable contribution that occurs when the equatorially oriented hydroxyl group at C4 of α(1−3) mannose of **1** is replaced with an axially oriented hydroxyl group at C4 as in galactose. In Gal6, on the other hand, the axially oriented hydroxyl group at C4, in addition, impairs the binding further which becomes evident from a comparison of its affinity with that of 4deoxy6 (**13**). This in turn indicates that the change in configuration at the C4 locus of α(1−6) mannose is not tolerated.

Among the derivatives of the trimannoside, 6deoxy3 (**15**) was completely inactive. Consequently, the indispensability of the C6 hydroxyl group of mannose at the α(1−3) arm is obvious. This observation highlights again that the α(1−3) mannose occupies the primary binding site of artocarpin, unlike ConA and DGL, where the α(1−6) Man is complementary to their monosaccharide binding site. The affinities of 3deoxy3 (**9**) and 3deoxy6 (**10**) are about 7- and 17-fold lower, respectively, while the binding enthalpies are reduced by 32.0 and 33.7 kJ mol^{-1}, respectively, implicating the C3 hydroxyl groups of mannose residues on both the arms of the trimannoside just as they are involved for the interaction with ConA and DGL. Likewise, the C6 hydroxyl group of the α(1−6) mannose (**14**) is also implicated in the interaction with the corresponding loci on artocarpin. Poor binding of 2deoxyc (**7**) and 4deoxyc (**12**) is consistent with the essential requirement of the hydroxyl groups at C2 and C4 of the

Table 1: Thermodynamic Parameters of Binding of Glc, Gal, and Monodeoxy Analogues to Artocarpin in Phosphate-Buffered Saline at pH 7.4[a]

sugar[b]	temp (K)	K_b (M^{-1})	$-\Delta H_b°$ (kJ mol^{-1})	$-\Delta G_b°$ (kJ mol^{-1})	$\Delta S_b°$ (J K^{-1} mol^{-1})	relative affinity[c]
trimannoside (**1**)	281.00	20100 ± 900	44.8 ± 1.42	23.15 ± 0.10	−77.02 ± 5.45	1
	293.20	9000 ± 500	46.10 ± 1.48	22.19 ± 0.09	−81.53 ± 6.13	
Glc3 (**2**)	280.80	1100 ± 56	21.2 ± 1.46	16.35 ± 0.12	−17.27 ± 5.47	0.05
	292.90	630 ± 41	23.12 ± 1.56	15.70 ± 0.11	−25.28 ± 6.02	
Glc6 (**3**)	281.00	5960 ± 490	16.0 ± 0.14	20.31 ± 0.19	15.31 ± 1.18	0.30
	293.00	2950 ± 410	18.13 ± 0.21	19.46 ± 0.21	4.65 ± 1.09	
Gal3 (**4**)	280.90	1370 ± 79	12.1 ± 0.36	16.86 ± 0.14	16.95 ± 1.77	0.07
	293.00	720 ± 60	13.51 ± 0.43	16.03 ± 0.12	8.62 ± 1.95	
Gal6 (**5**)	281.00	885 ± 32	20.0 ± 0.98	15.85 ± 0.08	−14.75 ± 3.78	0.04
	293.20	540 ± 19	21.93 ± 1.09	15.34 ± 0.07	−22.38 ± 4.01	
2deoxy3 (**6**)	281.00	820 ± 47	14.5 ± 1.40	15.67 ± 0.13	4.16 ± 0.45	0.04
	292.90	510 ± 33	16.00 ± 1.49	15.18 ± 0.15	−2.79 ± 0.59	
2deoxyc (**7**)	280.90	880 ± 38	15.0 ± 1.07	15.83 ± 0.10	2.95 ± 0.19	0.04
	293.10	545 ± 21	16.57 ± 1.19	15.35 ± 0.08	−3.96 ± 0.26	
2deoxy6 (**8**)	280.90	1650 ± 85	22.7 ± 0.80	17.30 ± 0.12	−19.22 ± 3.30	0.08
	293.20	910 ± 73	24.10 ± 0.98	16.61 ± 0.16	−25.55 ± 3.35	
3deoxy3 (**9**)	280.80	3000 ± 190	10.7 ± 0.78	18.69 ± 0.18	28.45 ± 3.43	0.13
	293.00	1620 ± 88	12.26 ± 0.87	18.00 ± 0.21	19.80 ± 3.52	
3deoxy6 (**10**)	280.90	1220 ± 58	20.9 ± 0.64	16.59 ± 0.11	−15.34 ± 2.65	0.06
	293.00	710 ± 38	22.29 ± 0.73	15.99 ± 0.10	−21.53 ± 2.78	
4deoxy3 (**11**)	280.80	1210 ± 140	5.2 ± 0.91	16.57 ± 0.26	40.49 ± 4.53	0.06
	292.90	660 ± 100	7.10 ± 0.99	15.81 ± 0.29	26.74 ± 4.96	
4deoxyc (**12**)	280.70	1250 ± 29	19.3 ± 0.80	16.64 ± 0.05	−9.47 ± 3.05	0.06
	293.20	730 ± 10	21.23 ± 0.92	16.07 ± 0.09	−17.49 ± 3.66	
4deoxy6 (**13**)	280.80	2160 ± 86	25.8 ± 0.63	17.93 ± 0.09	−28.02 ± 2.59	0.11
	293.00	1080 ± 70	27.15 ± 0.76	17.02 ± 0.10	−34.42 ± 2.67	
6deoxy6 (**14**)	281.00	1640 ± 47	20.9 ± 0.64	17.29 ± 0.07	−12.84 ± 2.51	0.08
	293.10	930 ± 27	22.14 ± 0.73	16.66 ± 0.08	−18.57 ± 2.71	
6deoxy3 (**15**)	281.00	NB[d]	NB	NB	NB	NB
MeαMan	280.50	2500 ± 91	28.2 ± 0.84	18.24 ± 0.08	−35.22 ± 2.42	0.12
	293.50	1864 ± 63	28.77 ± 0.91	18.34 ± 0.12	−35.58 ± 2.55	

[a] A plot of $\Delta H_b°$ vs $T\Delta S°$ yields a linear plot with a slope of 1.11 and a correlation coefficient of 0.96. [b] As in Figure 1. [c] Relative affinity for the interaction of artocarpin with mannotriose and its analogues. [d] NB, no binding observed at 10 mM protein binding sites and 120 mM ligand.

Table 2: Thermodynamic Parameters Derived from the Titration of Artocarpin at pH 7.4 with Saccharides at 280 ± 1 K

carbohydrate	$-\Delta H_b°$ (kJ mol^{-1})	$-\Delta G_b°$ (kJ mol^{-1})	$\Delta\Delta H$ (kJ mol^{-1})	$\Delta\Delta G$ (kJ mol^{-1})
trimannoside (**1**)	44.80	23.15	−	
α(1−3) 2-deoxy (**6**)	6.30	15.67	38.50	7.5
core 2-deoxy (**7**)	7.00	15.83	37.80	7.3
α(1−6) 2-deoxy (**8**)	10.20	17.30	34.60	5.9
α(1−3) 3-deoxy (**9**)	12.80	18.69	32.00	4.5
α(1−6) 3-deoxy (**10**)	11.10	16.59	33.70	6.6
α(1−3) 4-deoxy (**11**)	10.00	16.57	34.80	6.6
core 4-deoxy (**12**)	14.20	16.64	30.6	6.5
α(1−6) 4-deoxy (**13**)	20.00	17.93	24.80	5.2
α(1−6) 6-deoxy (**14**)	16.20	17.29	28.60	5.9
α(1−3) 6-deoxy (**15**)	NB[a]	NB	NB	NB

[a] NB, no binding.

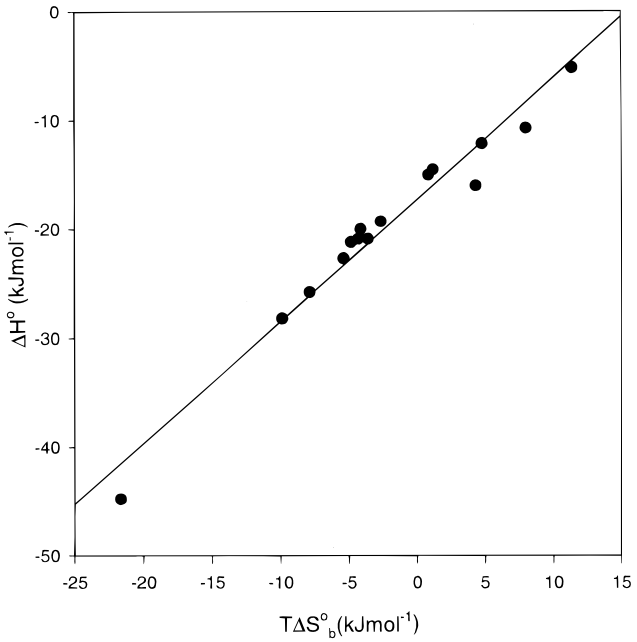

FIGURE 3: Plot of $\Delta H°$ vs $T\Delta S_b°$ for the binding of trimannoside and its Glc, Gal, and deoxy analogues at 281 K. The plot shows a linear relationship with a slope of 1.11 and a correlation coefficient of 0.96.

disubstituted mannose of **1** for the reaction with artocarpin. The fact that the replacement of the hydroxyl group with a deoxy group in any of the mannopyranosyl residues of the trimannoside ligand results in dramatic losses in binding enthalpies suggests that all the mannopyranosyl residues must interact simultaneously with their corresponding subsites in the combining site of artocarpin.

The thermodynamics of the interaction of saccharide ligands with a few lectins have been studied over the past decade. For most lectins, in general, it has been observed that they recognize only a few determinants even in their most complementary ligand(s). Noninvolvement of many of the hydroxyl groups in the binding reactions with lectins has led to the proposal of hypotheses about the importance of nonpolar interaction as the dominant force in protein−sugar reactions. Artocarpin, therefore, is unique among lectins, requiring the participation of all the hydroxyl groups of its ligand. These studies also indicate that hydrogen bonding plays a dominant role in the stabilization of its complex with

its complementary ligand. The example of artocarpin also underscores the fundamental differences in the nature of forces involved in its recognition of the complementary ligand vis a vis other lectins

As only a few hydroxyl groups directly participate in lectin−sugar interactions, it is sufficient for lectins to have their combining site as shallow indentations on their surface. Consequently, most lectins recognize sugar ligands through one or two of their edges (*31*, *32*). Interestingly, as all of the hydroxyl groups of trimannoside are recognized by artocarpin, the lectin recognizes either directly or through the solvent several edges of the ligand in contrast to the other lectins studied to date. Moreover, these features also prompt us to suggest that the combining site of artocarpin can be considered to be deep pocket as compared to the lectins described so far (*12*, *13*)

In conclusion, the thermodynamic description of the binding of trimannoside and a series of monodeoxy analogues of the same to artocarpin by isothermal titration calorimetry indicates that all of the hydroxyl groups of **1** participate in binding to the lectin, a feature quite distinct and unprecedented for this class of proteins. The results are thus consistent with a deeper combining site cleft in artocarpin as compared to shallow combining regions reported so far in the other lectins.

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