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Recognition of septanose carbohydrates by concanavalin A†

Steve Castro, Michael Duff, Nicole L. Snyder, Martha Morton, C. V. Kumar* and Mark W. Peczuh*

Department of Chemistry, The University of Connecticut, 55 North Eagleville Road U-3060, Storrs, CT, 06269, USA. E-mail: mark.peczuh@uconn.edu; Fax: +1 860-486-2981; Tel: +1 860-486-1605

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The ability of the jack bean lectin concanavalin A (ConA) to bind seven membered ring (septanose) monosaccharides has been investigated by isothermal titration calorimetry (ITC) and saturation transfer difference (STD) NMR spectroscopy.

A number of biologically important associations are mediated by protein–carbohydrate interactions. Examples that are often cited include cell–cell interactions in normal and diseased states,[1] bacterial and viral invasion into cells,[2] and tissue development.[3] Intracellular events such as editing of protein folding[4] and cell signalling[5] are also mediated by protein–carbohydrate interactons. The protein hosts of the carbohydrate ligands often demonstrate a high degree of selectivity for the anomeric configuration and conformation of their respective guests despite the modest affinities that are characteristic of protein–carbohydrate interactions. Affinity in these systems is governed by the number of individual associating groups[6] in concert with solvation/desolvation of ligands, van der Waals interactions, and hydrogen bonding complementarity between the protein and the ligand. Although typical investigations of protein–carbohydrate interactions have focused on pyranose carbohydrates as ligands, we became interested in answering the question, "can an unnatural seven membered ring (septanose) sugar be bound by a natural lectin?"

The recent introduction of expanded (or homologated) nucleic acids[7] and peptides[8] illustrates a general strategy toward the development of novel biomolecule analogs inspired by their natural counterparts. These unnatural structures are interesting because of their potential to interact with biological systems in a rational manner or in defining completely new systems.[9] Despite the conceptual simplicity of the homologation strategy, a systematic investigation of carbohydrates using this approach has remained relatively undeveloped.[10,11] As part of a research program concerned with the preparation and analysis of seven membered ring (septanose) carbohydrates,[12–14] we have discovered that the jack bean lectin concanavalin A (ConA) selectively binds β-septanosides in contrast to its natural selectivity for α-pyranoside ligands. The thermodynamic[15] and structural[16,17] characterization of ConA–carbohydrate complexation made ConA an attractive model system for the investigation of protein–septanose interactions. The results reported here provide direct evidence for the ability of natural proteins to bind unnatural septanose monosaccharides.

Binding of ConA to carbohydrates **1–7**, shown in Fig. 1, was monitored by ITC. Titration of ConA with substrates **1**, **2**, **6**, and **7** indicated exothermic binding, while substrates **3–5** showed no change in enthalpy. The raw data were corrected for heats of dilution, and the binding parameters were obtained by fitting the data to a single binding site model. The binding constants measured for methyl α-D-mannoside (**1**) and methyl α-D-glucoside (**2**) are characteristic of protein–carbohydrate interactions[18] and in good agreement with the reported values (Table 1).[15a] While the possibility of an enthalpically neutral binding event between α-septanosides **3–5** and ConA is formally possible, we consider it unlikely based on the available data. The β-septanosides **6** and **7** indicated binding affinities that were 15–20 times weaker than methyl α-D-mannoside and 4–5 times weaker than methyl α-D-glucoside (**2**); the ITC binding data for the ConA–**6** interaction is shown in Fig. 2. Although of low affinity, this is the first demonstration of the binding of an unnatural ring expanded carbohydrate by ConA.

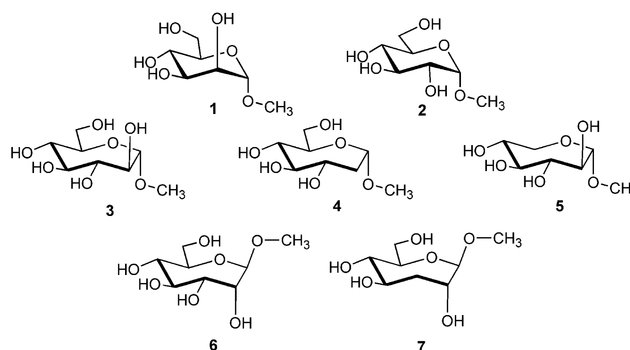

Fig. 1 Methyl α-pyranosides (**1**, **2**), methyl α-septanosides (**3–5**), and methyl β-septanosides (**6**, **7**).

According to the data in Table 1, association of ConA with **6** and **7** was governed largely by the $T\Delta S$ term. This is in contrast to association of ConA with **1** and **2** where the ΔH term was dominant. One explanation of this switch could be that because pyranoses and septanoses are of nearly equal dynamic flexibility,[14] the entropic contribution involves release of water molecules in going from the free to the bound state. This would include differential solvation/desolvation of the binding pocket or of the ligands. For example, the septanoses have a larger volume than pyranoses and an additional hydroxyl group. The stoichiometry (N) of ligands:binding sites for **6** and **7** also deviated from the expected value of one.[19] On the other hand, the β-septanosides **6** and **7** showed a high degree of selectivity (~400 : 1 or greater) with respect to the corresponding α-anomers **3–5**. This observation implied that ConA bound **6** and **7** at a discrete binding site rather than as a non-specific association. The discrepancies in thermodynamic parameters associated with the β-septanosides were likely to have been related to the low affinity of the interaction and will be discussed more below.

We next investigated the ConA–septanoside complexes by STD NMR spectroscopy[20,21] to corroborate the ITC data and to collect more specific information about the nature of the interaction. STD has been a useful tool for characterizing protein–ligand interactions; it allows observation of NMR

† Electronic supplementary information (ESI) available: STD NMR measurement details, STD spectra, NMR data, and ITC measurement details and data. See DOI: 10.1039/b509243d

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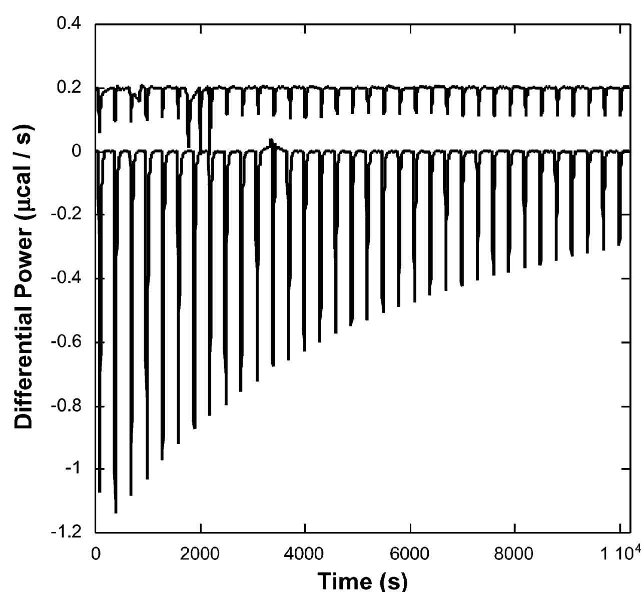
Table 1 Thermodynamics of binding (298 K) for ConA with methyl glycosides **1–7**[a]

Entry	Ligand	K_a/M^{-1}	N	ΔG/kcal mol^{-1}	ΔH/kcal mol^{-1}	$T\Delta S$/kcal mol^{-1}
1	**1**	$7.9 \pm 1.0 \times 10^3$	0.83 ± 0.18	-5.5 ± 0.1	-4.5 ± 0.03	-0.11 ± 0.2
2	**2**	$2.0 \pm 0.2 \times 10^3$	0.87 ± 0.03	-4.5 ± 0.03	-3.5 ± 0.9	0.98 ± 0.2
3	**3**	NB	—	—	—	—
4	**4**	NB	—	—	—	—
5	**5**	NB	—	—	—	—
6	**6**	$5.2 \pm 0.3 \times 10^2$	3.2 ± 0.9	-3.7 ± 0.1	-0.83 ± 0.1	2.9 ± 0.2
7	**6**	$4.5 \pm 0.7 \times 10^2$	1[b]	-3.6 ± 0.09	-2.7 ± 0.2	0.95 ± 0.3
8	**7**	$3.9 \pm 0.5 \times 10^2$	4.4 ± 0.4	-3.6 ± 0.05	-0.72 ± 0.1	2.8 ± 0.2
9	**7**	$4.8 \pm 2.0 \times 10^2$	1[b]	-3.6 ± 0.3	-2.4 ± 0.8	1.2 ± 1.1

[a] [ConA] was between 0.200–0.300 mM and [**1–7**] were between 15–30 mM (see the electronic supplementary information, ESI†). ConA was dimeric under the experimental conditions. Buffer: 50 mM 3,3-dimethylglutarate pH 5.2, 250 mM NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$. [b] Data were fitted with the ligand : ConA stoichiometry (N) manually fixed at one.



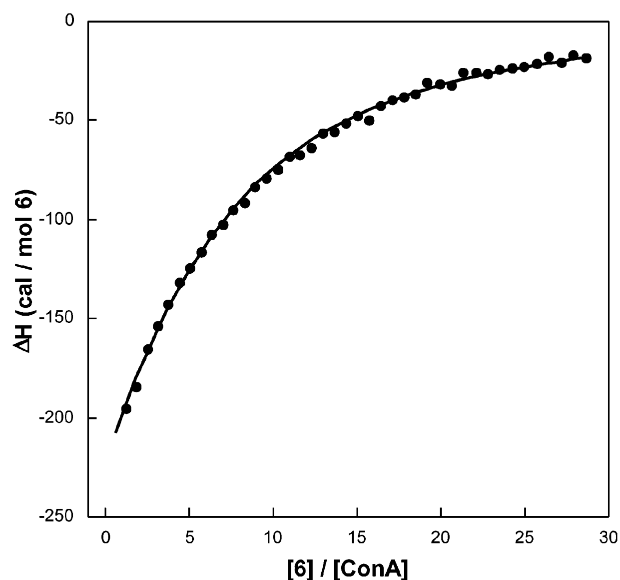


Fig. 2 Calorimetric titration of ConA (0.200 mM) with **6** (25.0 mM) at 298 K. See Table 1 for complete experimental conditions.

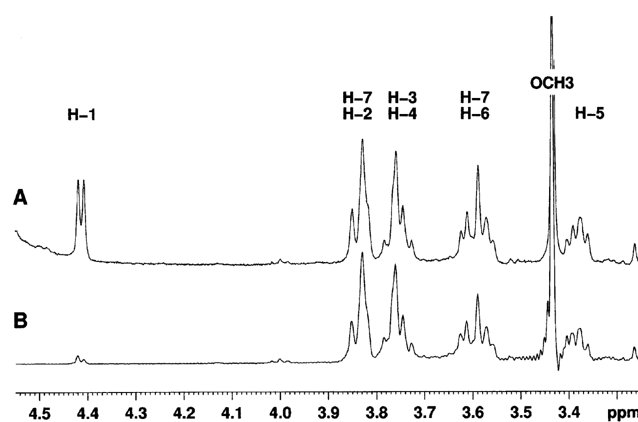


Fig. 3 (A) NMR spectra of **6** (8 mM) in the presence of ConA (100 µM) (50 mM CD_3CO_2D pH 4.7, 250 mM NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$ in D_2O) and (B) STD spectrum of the same complex.

signals that arise through selective transfer of magnetization from the protein to *bound* ligands. A sample of methyl β-D-*glycero*-D-guloseptanoside (**6**) (8 mM) in the presence of ConA (100 µM) showed STD signals indicative of binding (Fig. 3). *H*2 to *H*7 (septanose numbering) and the anomeric methoxy group were all in contact with ConA while the weak *H*1 signal suggested that it was not in close contact with the protein. Similar STD spectra were obtained when either the related methyl β-3-deoxy-D-*glycero*-D-guloseptanoside (**7**) or methyl α-D-glucoside (**2**) (as a control) was used as ligand. Additional control STD experiments using methyl α-D-*glycero*-D-idoseptanoside (**3**) or methyl β-D-glucoside as ligand gave rise to significantly attenuated STD signals suggestive of no binding by ConA to these ligands (see electronic supplementary information, ESI†).

An STD NMR competition experiment was conducted to confirm that the β-septanoside ligands were bound by ConA in a way that is analagous to α-pyranosides. Fig. 4 shows STD spectra of: (A) 4 mM methyl α-D-mannoside (**1**); (B) 4 mM β-3-deoxy-D-*glycero*-D-guloseptanoside (**7**); and (C) a 1 : 1 mixture of **1** and **7** (4 mM each) all in the presence of ConA (100 µM). The appearance of STD signals in each experiment suggested ligand binding by ConA. The STD spectrum of the competition experiment (Fig. 4C) corresponded best with methyl α-D-mannoside (**1**), suggesting that the β-septanosides were in fact being bound in the same pocket of ConA as α-pyranosides. Overall, the results of the STD experiments reinforced the pattern of recognition from the ITC experiments and also demonstrated that β-septanosides were recognized at the ligand (α-pyranoside) binding site of ConA.

The results of the STD competition experiment prompted us to reconsider the thermodynamic parameters obtained by ITC. The low affinities of ConA for **6** and **7** measured resulted in a c parameter ($c = 0.1$) that probably compromised the accuracy in the determination of stoichiometery (N) and underestimated the magnitude of the ΔH values.[22,23] By fixing the N value to one (Table 1, entries 7, 9) in the analysis of the ITC data for **6** and **7**, a different picture for the thermodynamics of binding emerged. We argue that fixing the N value was valid based on the inherent selectivity (β) amongst the methyl septanosides **3–7** and because they are bound in the same pocket of ConA

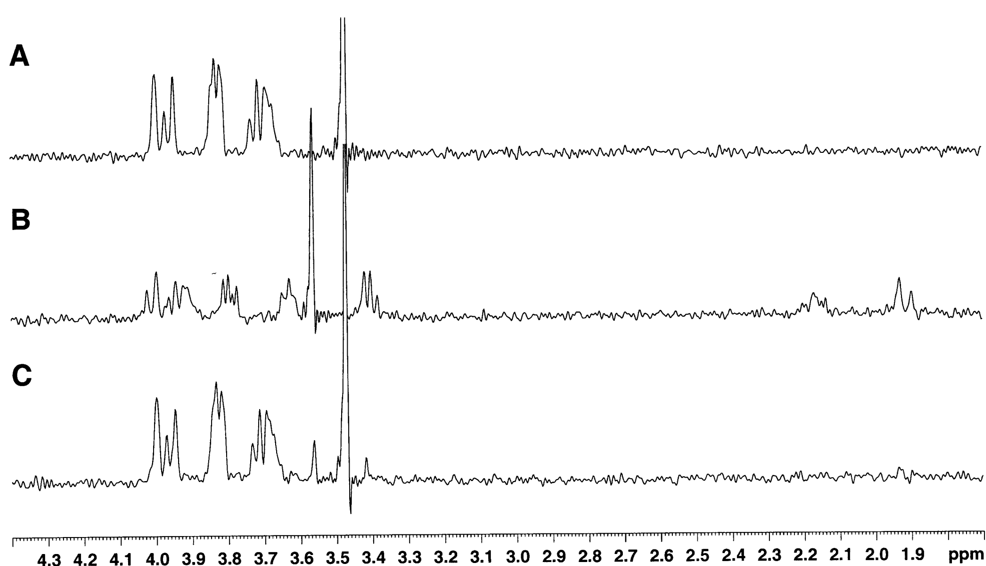


Fig. 4 STD NMR spectra of: (A) **1** (4 mM); (B) **7** (4 mM); and (C) **1** (4 mM) and **7** (4 mM), all in the presence of ConA (100 μM) (50 mM CD_3CO_2D pH 4.7, 250 mM NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$ in D_2O).

as pyranosides. The association constants remained similar to those from the floating fits under this restricted computational treatment.[24] Additionally, the ΔH terms approached values that are similar to pyranosides, again implying that ConA binds septanosides in a manner similar to pyranosides. Specifically, it suggested to us that the β-septanosides were making a collection of contacts with ConA reminiscent of α-pyranosides. It is important to emphasize that the approximation made here simply informs the original question on whether unnatural septanose monosaccharides can be bound by ConA.[25]

While the specific structural details that describe the association observed here have yet to be elucidated, our interpretation of the binding for methyl glycosides **1–7** uses knowledge about the conformation of each ligand and the ConA binding pocket defined by the known crystal structures. Pyranosides **1** and **2** are known to take up a $^4C_1$ conformation. We have recently reported that septanosides such as **3** and **6** adopt one conformation ($^{3,4}TC_{5,6}$ and $^{6,O}TC_{4,5}$, respectively) in solution.[14] The crystal structure of **4**, the 2-deoxy analog of **3**[26] also showed a $^{3,4}TC_{5,6}$ conformation. Manual overlays of **4** or **6** with the methyl α-D-glucoside (**2**) taken from the ConA–**2** crystal structure[16] show good overlap indicating that their conformations are similar. Further, all the ligands share the same stereochemistry from C3 to C6 (pyranose numbering)- the region that makes contact with ConA. Assuming a common binding orientation for pyranoses and septanoses, the β-septanosides are likely to be of lower affinity than α-pyranosides due to the mismatch between the expanded ring size and the binding pocket of ConA. As with pyranosides, consideration of steric interactions between the aglycon methyl group of the ligands and the leucine residue 99 (L99) of ConA may provide a rationale for the selectivity in binding β- rather than α-septanosides.

In summary, the data show that concanavalin A binds methyl β-D-septanosides with modest affinity and in preference to methyl α-D-septanosides. Further, methyl β-D-septanosides are bound competitively with the natural α-D-pyranoside ligands. This is the first direct evidence for this class of ring expanded carbohydrates being bound by a natural protein. Although the affinities for septanosides reported here are low relative to their pyranoside counterparts, the observation of binding broadens the scope of protein–carbohydrate interactions generally. Our current efforts include more detailed thermodynamic and structural characterization of the ConA–septanoside complexes and preparation of new ligands to be used in the development of structure–activity relationships based on the model for septanose binding described here.

Acknowledgements

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