Plasticity in the Primary Binding Site of Galactose/N-Acetylglucosamine-specific Lectins

Implication of the C–H–O Hydrogen Bond at the Specificity-determining C-4 Locus of the Saccharide in 4-Methoxygalactose Recognition by Jacalin and Winged Bean (Basic) Agglutinin I*

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It is currently believed that an unsubstituted axial hydroxyl at the specificity-determining C-4 locus of galactose is indispensable for recognition by galactose/N-acetylglucosamine-specific lectins. Titration calorimetry demonstrates that 4-methoxygalactose retains binding allegiance to the Moraceae lectin jacalin and the Leguminosae lectin, winged bean (basic) agglutinin (WBA I). The binding reactions were driven by dominant favorable enthalpic contributions and exhibited significant enthalpy-entropy compensation. Proton NMR titration of 4-methoxygalactose with jacalin and WBA I resulted in broadening of the sugar resonances without any change in chemical shift. The α- and β-anomers of 4-methoxygalactose were found to be in slow exchange with free and lectin-bound states. Both the anomers experience magnetically equivalent environments at the respective binding sites. The binding constants derived from the dependence of NMR line widths on 4-methoxygalactose concentration agreed well with those obtained from titration calorimetry. The results unequivocally demonstrate that the loci corresponding to the axially oriented C-4 hydroxyl group of galactose within the primary binding site of these lectins exhibit plasticity. These analyses suggest, for the first time, the existence of C–H–O-type hydrogen-bond(s) in protein–carbohydrate interactions in general and between the C-4 locus of galactose derivative and the lectins jacalin and WBA I in particular.

The recognition of sugar molecules by carbohydrate-specific proteins, lectins, involves the establishment of an organized set of interactions within the binding site. Hydrogen-bonding interactions are one of the most important factors of molecular recognition in lectin–sugar interactions, along with van der Waals forces, which, although rather weak (often contributing only a fraction of 1 kcal mol⁻¹ for each pair of atoms), are frequently numerous and together make a significant contribution to binding (1, 2). Despite the differences in the lectin folds and their modes of sugar binding, the specificity for the recognition of galactose is determined by interactions involving the C-4 locus of the saccharide (1–5). Stereochemical evidence is emerging for a distinct sugar binding specificity-dependent distribution of hydrogen bond donors vis-à-vis the acceptors in the combining site of lectins (4). The C-4 locus of the monosaccharide within the primary binding site of galactose/N-acetylglucosamine-specific lectins has hitherto been considered to be absolutely invariant.

Though belonging to different families, jacalin, a Moraceae member, and WBA I, a Leguminosae member, both are galactose/N-acetylglucosamine-specific lectins (6, 7). Whereas jacalin displays a β-prism tertiary structural fold wherein a unique post-translationally generated N-terminal glycin residue serves as a critical determinant of galactose specificity (8), WBA I contains a legume lectin fold (9). During the course of mapping and establishing the hydrogen bond donor-acceptor relationship of the primary combining site of galactose-specific lectins (10), unexpectedly, we have discovered that the 4-methoxy derivative of β-galactopyranoside (4-methoxygalactose) binds, with affinities comparable with that of methyl α-galactose, to the Moraceae lectin jacalin and the Leguminosae lectin WBA I but not to the related WBA II.²

Experimental Procedures

Materials—All reagents were of analytical or ultrapure grade. Methyl α-galactose was purchased from Sigma. 4-methoxygalactose was synthesized as described, and its purity was checked by melting point, thin-layer chromatography, and high resolution 1H-NMR at 250 MHz on a Bruker spectrometer (10). Deionized Milli-Q water was used for all studies.

Preparation and Analysis of Solutions—All protein as well as sugar solutions were prepared in 20 mM phosphate buffer (pH 7.2) containing 150 mM sodium chloride (PBS). Jacalin (6), WBA I (7, 10), and WBA II (12) were prepared by affinity chromatography as described. Their concentrations were measured spectrophotometrically using ε205 nm = 15.8, 9.37, and 7.7, respectively, determined by weight method as well as from amino acid sequence data. Jacalin is a tetramer, whereas WBA I and WBA II are dimers.

ITC Measurements and Analyses—The titration calorimetric measurements and analyses were performed with a Microcal⁳ Omega titration calorimeter as described (10, 13, 14). Aliquots of the sugar solution at 10–100 times the binding site concentration were added via a 250-μL rotating stirrer syringe to the lectin solution in the calorimetric cell under isothermal conditions. The dimensionless quantity c, a product of the binding constant K and the total concentration of macromolecule [M] in the cell, was >2.5 but <50, corresponding to a binding regime best suited for the most precise measurements of the binding stoichi-

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¹ The abbreviations used are: WBA, winged bean (basic) agglutinin; ITC, isothermal titration calorimetry; EEC, enthalpy-entropy compensation.

² Srinivas, V. R., Acharya, S., Rawat, S., Sharma, V., and Surolia, A. (2000) FEBS Lett. 474, 76–82.
omery, $K_a$ and enthalpy in a single ITC experiment. Heats of dilution of the sugar ligand were subtracted from the runs conducted with the lectins.

**NMR Measurements and Analyses**—NMR samples were prepared in PBS in D$_2$O, pH 7.2. The resultant protein concentration in the samples was 0.2 mM for jacalin, WBA I, and WBA II. The concentration of 4-methoxygalactose was varied from 0.4 to 3 mM. The 400 MHz 1H-NMR spectra of 4-methoxygalactose were recorded on a Bruker AMX-400 NMR spectrometer. The chemical shifts were referenced to the methyl signal of tetramethylsilane as internal standard. Water suppression was performed by the pre-saturation method. The assignment of 4-methoxy signal in the NMR spectra was performed as described (15). The NMR spectra of 4-methoxygalactose are in agreement with the $^4$C$_1$ pyranoid conformation (15, 16). Line broadening of resonances was measured at half-height of the resonance under observation after correction for magnetic field inhomogeneity. The line broadening of a small molecule such as 4-methoxygalactose, because of its binding to a macromolecule such as the lectin jacalin or WBA I, was treated according to the method of Swift and Connick (17). $T_m = (r_m + T_{m0})/[K_p][P]$, $T_{m0}$ is the reciprocal of net change in respective line width at half-height, $r_m$ is the residence time of the anomers in the protein binding site, $T_{m0}$ is the spin-spin relaxation time in the bound environment, $[P]$, is the total protein concentration, and $K_p$ ([β]/[α]) is the ratio of the anomers in equilibrium. A plot of reciprocal line broadening, $1/T_m$, as a function of 4-methoxygalactose concentration displayed linear dependence for both WBA I and jacalin. The negative intercept on the x axis gives the dissociation constant ($1/K_p$) for the anomer, and the y intercept yields the residence time $r_m$. In the slow exchange limit ($r_m>>T_{m0}$), as is the case here, the line broadening is governed by the exchange rate $1/r_{m0}$, which is equal to the dissociation rate constant $k_-$ of the anomer-lectin complex (17, 18).

**RESULTS AND DISCUSSION**

The ITC experiments directly detected the evolution of heats of binding when fixed aliquots of 4-methoxygalactose solution were added into either jacalin solution (Fig. 1A) or WBA I solution (Fig. 1C). These data, analyzed by iterated non-linear least squares fitting procedures, were found to best fit the simplest identical site model indicating that both the lectins bind to 4-methoxygalactose (Fig. 1B, 1D). The reactions were driven by dominant favorable enthalpic contributions (Table I). These results, contrary to the usual expectation of the indispensability of the unsubstiuted C-4 hydroxyl of the saccharide, clearly demonstrate the capability of 4-methoxygalactose to bind to both WBA I as well as jacalin. This reflects the existence of 4-methoxygalactose binding ability in galactose/N-acetylglactosamine-specific lectin members from at least two unrelated families. To test whether 4-methoxygalactose displays a promiscuous binding to other lectins with similar monosaccharide specificity, the binding of 4-methoxygalactose to WBA II, a dimeric acidic lectin sequentially and structurally similar to WBA I, was tested. The results of such an ITC experiment indicate no binding of 4-methoxygalactose to WBA II (Fig. 1E and F). That the non-binding of 4-methoxygalactose to WBA II was not due to loss of activity of WBA II was confirmed by testing the binding of 2 fucosyllactose to WBA II; the thermodynamic parameters obtained, $K_i = 1.0 \times 10^3$ and $\Delta H^{\circ} = -43.2$ kJ mol$^{-1}$ at 298.2 K, agreed very well with previously published values (12). In contrast, none of these galactose/N-acetylglactosamine-specific lectins, WBA I, WBA II, or jacalin, bound 4-methoxyglucose. The inherent inability of WBA II to recognize 4-methoxygalactose is perhaps due to dominant steric factors around its specificity-determining C-4 locus. The ability of 4-methoxygalactose to bind to WBA I as well as jacalin, together with its inability to bind to WBA II, is consistent with the formation of favorable interactions from the vicinity of the C-4 locus of 4-methoxygalactose with the corresponding binding site residues in WBA I and jacalin. It appears that the disposition of amino acid residues in the vicinity of the C-4 locus of 4-methoxygalactose within the binding sites of WBA I and jacalin permits the efficient binding of 4-methoxygalactose by WBA I and jacalin. That 3-methoxygalactose and 6-methoxygalactose do not bind to either WBA I (10) or jacalin indicates that this unusual binding of 4-methoxygalactose by WBA I and jacalin is due to specific interactions. In addition, WBA I–4-methoxygalactose as well as jacalin–4-methoxygalactose complexes saturated with 4-methoxygalactose did not bind to a molar excess of exogenously added methyl-α-galactose, suggesting that 4-methoxygalactose was not bound to a site other than the primary binding site of either jacalin or WBA I.

The temperature dependence of the enthalpy ($\Delta H_{b}^0$) (Fig. 1G) and entropy ($\Delta S_{b}^0$) (Fig. 1H) was linear for the binding of 4-methoxygalactose to both jacalin and WBA I. There is almost no temperature dependence of the binding free energy (i.e. $\Delta G_{b}^0 \approx 0$) (Fig. 1I) within the temperature range examined because of significant enthalpy-entropy compensation (EEC) (Fig. 1J). EEC apparently masks the differences in binding.

3 C. P. Swaminathan and A. Surolia, unpublished results.
thermodynamics (14, 19). A close examination of the EEC plot (Fig. 1J) reveals that the effect of EEC, in terms of the slope of the linear plots, is nearly the same for 4-methoxygalactose binding both to jacalin and WBA I. The y intercept (i.e. a position in the EEC plot where $-T\Delta S_b^0$ is zero) of the EEC plot provides a measure of the condition at which all contributions from the enthalpic components proceed entirely to the free energy of the system, without any net change in entropic losses or gains. Thus, for enthalpically driven systems, such as the energy of the system, without any net change in entropic losses, difference in binding of 4-methoxygalactose to jacalin and WBA I, the difference in reciprocal line broadening as a function of 4-methoxygalactose concentration yielded $K_b$ values of $7.4 \times 10^3$ and $7.3 \times 10^3$ for the binding of $\alpha$- and $\beta$-anomers, respectively, of 4-methoxygalactose to WBA I, whereas the $K_b$ for the jacalin–4-methoxygalactose complex was $1.5 \times 10^4$ (Fig. 2D). These values are in the range of titration calorimetric data (Table I). The ITC and NMR results together demonstrate unequivocally that the C-4 locus of galactose can tolerate substitution and yet not disrupt the specific carbohydrate binding ability of jacalin and WBA I, thus throwing light on the plasticity of their primary combining sites. Hence the binding site should be sufficiently flexible to accommodate this plasticity, which permits a promiscuous recognition of both galactose and 4-methoxygalactose.

These studies point to the existence of a C–H–O hydrogen bond between the 4-methoxy group of 4-methoxygalactose and the corresponding loci in the binding site of jacalin and WBA I. The presence of oxygen atoms in a large majority of molecules raises the possibility that the C–H–O hydrogen bond is widespread though not identified in many cases (20–22). However, x-ray and neutron diffraction studies have shown that crystals of various organic molecules and biomolecules exhibit close C–H–X contacts (where X is an electronegative atom, in most cases oxygen) that show all the stereoechemical hallmarks of hydrogen-bonds (21). Recently, C–H–O interactions in collagen triple helix (23), DNA–protein complex (24), thrombin-inhibitor complex (25), trypanothione reductase-trypanothione disulﬁde complex (26), active sites of serine hydrolases (27), and helices involving proline residues (28) have also been identiﬁed. Cytosine-rich intercalated DNA quadruplexes not only contain intra-cytidine C–H–O hydrogen bonds but also display a systematic intermolecular C–H–O hydrogen bonding network between the deoxyribose sugar moieties of antiparallel backbones in the four-stranded molecule (29). More recently, C–H–O hydrogen bonds have been found in the minor grooves of A-tracts in DNA double helices (30), and, as a caveat, in cubaneac-
Thus substantiate the existence of C–H–O hydrogen bond(s) in the vicinity of the specificity-determining C-4 loci of the bound saccharide in the complexes of WBA I–4-methoxygalactose (Fig. 3B) and jacalin–4-methoxygalactose (Fig. 3D). These C–H–O hydrogen bonding interactions involved in the recognition of 4-methoxygalactose by WBA I and jacalin appear to be stronger in magnitude (Table I) than those involved in the binding of galactose with WBA I (10) and jacalin. The examples of 4-methoxygalactose–WBA I/jacalin interactions thus suggest that C–H–O hydrogen bonds in lectin–sugar interactions could contribute at least as significantly to the binding reaction as other hydrogen bonds.

Aside from the main chain carbonyl of Thr-210 in WBA I at a distance of 4.39 Å from the methyl group oxygen at the C-4 locus of the modeled 4-methoxy-o-α-galactopyranoside, no atom other than the cognate binding site residues was present within 5.5 Å in the primary binding sites of WBA I and jacalin (11). The absence of nonpolar residues around the C-4 loci of methyl-α-galactose-bound complexes of WBA I and jacalin provide grounds to believe that the interaction of the 4-methoxy group of 4-methoxygalactose with the binding sites of WBA I and jacalin is not of a nonpolar nature. This is also supported by the observation that these binding reactions are predominantly enthalpically driven (Table I). Moreover, the root mean square deviation of Cα atoms of residues around the binding sites of native WBA I compared with those of WBA I complexed with methyl-α-galactose is less than 0.5 Å, suggesting the absence of significant conformational changes upon sugar binding.

In conclusion, we have presented here for the first time evidence for the existence in lectin–carbohydrate recognition of C–H–O hydrogen bond(s) in the vicinity of the specificity-determining C-4 locus of the saccharide 4-methoxygalactose and the lectins WBA I and jacalin.

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REFERENCES

1. Goldstein, I. J., and Hayes, C. E. (1978) Adv. Carbohydr. Chem. Biochem. 33, 127–340
2. Lis, H., and Sharon, N. (1998) Chem. Res. 98, 637–674
3. Sharma, V., and Surolia, A. (1997) J. Mol. Biol. 267, 433–445
4. Elgavish, S., and Shaiin, B. (1998) Trends Biochem. Sci. 22, 462–467
5. Vijayan, M., and Chandra, N. (1999) Curr. Opin. Struct. Biol. 9, 707–714
6. Sastry, M. V., Banerjee, P., Patangali, S. R., Swamy, M. J., Swarnalatha, G. V., and Surolia, A. (1996) J. Biol. Chem. 261, 11726–11733
7. Khan, M. I., Sastry, M. V., and Surolia, A. (1996) J. Biol. Chem. 261, 3013–3019
8. Sankaranarayanan, R., Sekar, K., Banerjee, R., Sharma, V., Surolia, A., and Vijayan, M. (1996) Nat. Struct. Biol. 3, 596–603
9. Prabu, M. M., Sankaranarayanan, R., Puri, K. D., Sharma, V., Surolia, A. (1998) J. Mol. Biol. 276, Vijayan, M., and Suguna, K. 2, 787–796
10. Swaminathan, C. P., Gupta, D., Sharma, V., and Surolia, A. (1997) Biochemistry 36, 13428–13434
11. Humphrey, W., Dalke, A., and Schulten, K. (1996) J. Mol. Graph. 14, 33–38
12. Srinivas, V. R., Singha, N. C., Schwarz, F. P., and Surolia, A. (1998) Carbohydr. Lett. 3, 129–136
13. Wiseman, T., Williston, S., Brandts, J. F., and Lin, L. N. (1989) Anal. Biochem. 179, 131–137
14. Swaminathan, C. P., Surolia, N., and Surolia, A. (1998) J. Am. Chem. Soc. 120, 5153–5159
15. Rithbone, B. R., Stephen, A. M., and Pachler, K. G. R. (1971) Carbohydr. Res. 20, 357–367
16. Casu, B., Reggiani, M., Gallo, G. G., and Vigevani, A. (1968) Tetrahedron 24, 803–821
17. Swift, J. J., and Connick, R. E. (1962) J. Chem. Phys. 37, 307–320
18. Sastry, M. V., Swamy, M. J., and Surolia, A. (1988) J. Biol. Chem. 263, 14826–14831
19. Cooper, A. (1999) Curr. Opin. Chem. Biol. 3, 557–563
20. Taylor, R., and Kennard, O. (1982) J. Am. Chem. Soc. 104, 5063–5070
21. Desiraju, G. R. (1986) Acc. Chem. Res. 29, 441–449
22. Derewenda, Z. S., Lee, L., and Derewenda, U. (1995) J. Mol. Biol. 252, 28486

Plasticity in Lectin–Sugar Binding via C–H–O Hydrogen Bonds

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23. Bella, J., and Berman, H. M. (1996) \textit{J. Mol. Biol.} \textbf{264}, 734–742
24. Mandel-Gutfreund, Y., Margalit, H., Jernigan, R. L., and Zhurkin, V. B. (1998) \textit{J. Mol. Biol.} \textbf{277}, 1129–1140
25. Engh, R. A., Brandstetter, H., Sucher, G., Eichinger, A., Baumann, U., Bode, W., Huber, R., Pull, T., Rudolph, R., and von der Saal, W. (1996) \textit{Structure} \textbf{4}, 1353–1362
26. Bond, C. S., Zhang, Y., Berriman, M., Cunningham, M. L., Fairlamb, A. H., and Hunter, W. N. (1999) \textit{Structure Fold. Des.} \textbf{7}, 81–89
27. Derewenda, Z. S., Derewenda, U., and Kobos, P. M. (1994) \textit{J. Mol. Biol.} \textbf{241}, 83–93
28. Chakrabarti, P., and Chakrabarti, S. (1998) \textit{J. Mol. Biol.} \textbf{284}, 867–873
29. Berger, I., Egli, M., and Rich, A. (1996) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{93}, 12116–12121
30. Ghosh, A., and Bansal, M. (1999) \textit{J. Mol. Biol.} \textbf{284}, 1149–1158
31. Kudava, S. S., Craig, D. C., Nangia, A., and Desiraju, G. R. (1999) \textit{J. Am. Chem. Soc.} \textbf{121}, 1036–1044
32. Steiner, T., and Desiraju, G. R. (1998) \textit{J. Chem. Soc. Chem. Commun.} 891–892
33. Desiraju, G. R., Kashino, S., Coombs, M. M., and Glusker, J. P. (1993) \textit{Acta Crystallogr. Sect. B Struct. Crystallogr.} \textbf{49}, 880–892
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