Research Communication

Distortion of the Ligand Molecule as a Strategy for Modulating Binding Affinity: Further Studies Involving Complexes of Jacalin with β-Substituted Disaccharides

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Abstract

Crystal structures of jacalin in complex with GlcNAc *β*-(1,3) Gal-*β*-OMe and Gal *β*-(1,3) Gal-*β*-OMe have been determined. The binding of the ligands to jacalin is similar to that of analogous α-substituted disaccharides. However, the *β*-substituted *β*-(1,3) linked disaccharides get distorted at the anomeric center and the glycosidic linkage. The distortion results in higher internal energies of the ligands leading to lower affinity to the lectin. This confirms the possibility of using ligand distortion as a strategy for modulating binding affinity. Unlike in the case of *β*-substituted monosaccharides bound to jacalin, where a larger distortion at the anomeric center was observed, smaller distortions are distributed among two centers in the structures of the two *β*-substituted *β*-(1,3) linked disaccharides presented here. These disaccharides, like the unsubstituted and α-substituted counterparts, bind jacalin with the reducing Gal at the primary binding site, indicating that the lower binding affinity of *β*-substituted disaccharides is not enough to overcome the intrinsic propensity of Gal *β*-(1,3) Gal-based disaccharides to bind jacalin with the reducing sugar at the primary site. © 2017 IUBMB Life, 69(2):72–78, 2017

Keywords: *T-antigen binding protein; galactose specific lectin; β-prism I fold; ligand distortion; reducing and nonreducing sugars*

Introduction

The structure determination of jacalin in this laboratory established *β*-prism I domain as a lectin fold (1). The lectin has since been thoroughly studied (2). The tetrameric glycosylated lectin, which has one sugar binding site on each subunit, is galactose specific at the monosaccharide level. At the disaccharide level, the lectin specifically binds with high affinity to the tumor associated T-antigen (Gal *β*-(1,3) GalNAc) (3,4), thus endowing the protein with diagnostic potential (5). The X-ray structure analysis of jacalin also indicated the role of an interaction of the bound sugar with an amino terminus generated by post-translational proteolysis of a single chain precursor in endowing the lectin with galactose specificity, thus identifying post-translational proteolysis as a strategy for generating ligand specificity (1). The post-translational proteolysis results in a subunit made up of a 133 amino acid residue-long α-chain and a 20 residue-long *β*-chain, held together as a single globular unit. In the homologous mannose-specific *β*-prism I lectins, each subunit is made up of a single polypeptide chain in the absence of proteolysis (6). The presence of a free amino group generated by post-translational proteolysis is now well established as a characteristic feature of the primary binding site of galactose specific *β*-prism I fold lectins like jacalin (7). The presence of aromatic residues also differentiates the binding site of such lectins from those of homologous mannose-specific lectins.

The extended binding site of jacalin was fully characterized by the middle of the last decade (4,8,9). The plasticity of the binding site was further explored subsequently using molecular dynamics simulations (10). However, two specific

Abbreviations: *Gal, galactose; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NaCl, sodium chloride; PDB, Protein data bank; T-antigen, Thomsen–Friedenreich antigen (Gal β-(1,3) GalNAc)*

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issues remained unexplored. One had to do with the mode of binding of β-substituted sugars to jacalin. *Prima facie*, it appeared that a β-substitution of galactose at the primary binding site of the lectin would lead to unacceptable steric clashes (8). The issue was further explored through crystallographic studies of the interaction of jacalin with α-substituted as well as β-substituted derivatives of galactose involving methyl, *p*-nitrophenyl and 4-methylumbelliferyl groups (11). Interactions of the substituent with the protein remain essentially the same irrespective of the anomeric nature of the substitution. This is made possible by a distortion of the sugar ring in β-galactosides. This distortion leads to a reduction in the overall binding energy of the β-substituted sugars. Distortion of the ligand is thus used as a strategy for modulating the affinity (11). The study referred to above involved galactose derivatives. However, biologically relevant interactions at the combining site of jacalin primarily involve β-(1,3) linked disaccharides. Structural studies presented here suggest that the binding of β-substituted derivatives of such disaccharides to jacalin is facilitated by a combination of ring distortion and distortion at the glycosidic link, with a concomitant energy cost.

The second underexplored issue was concerned with the effect of linkage on the location of reducing and nonreducing sugars bound to jacalin. This issue was recently investigated using a combination of crystallographic and modeling approaches (12). The investigation indicated that a location with the reducing sugar at the primary binding site and the nonreducing sugar at the secondary binding site B is preferred for a β-(1,3) linked disaccharide. One with the nonreducing sugar at the primary site and the reducing sugar at a different secondary site is possible, but less likely. α-Substitution of a methyl group in the former arrangement would strengthen the binding. A β-substituted disaccharide can bind with this arrangement only with a distortion in the ligand molecule, but no such distortion is necessary if the nonreducing end is at the primary binding site. Thus, a β-substituted β-(1,3) linked disaccharide can bind jacalin in the preferred orientation with ligand distortion or in the less preferred orientation with no distortion in the ligand molecule. The former alternative is observed in the crystal structures of the jacalin complexes with β-substituted disaccharides reported here. Thus, the preference for the arrangement with the reducing sugar at the primary binding site appears to be strong enough to compensate for the energy loss associated with the distortion of the ligand.

Material and Methods

Purification and Crystallization

The protein was purified from jack fruit seed extract thorough a galactose crosslinked guar gum column using the procedure described earlier (13). The protein was dialyzed against 20 mM phosphate buffer (pH 7.3) containing 150 mM NaCl and 0.025% sodium azide to remove all the bound galactose. It was crystallized using vapor diffusion technique at 25 °C in conditions used in the previous studies (11,12,14). Briefly, equilibration of a 4 μL drop of 12 mg/mL protein in 10 mM HEPES buffer (pH 7.4), 150 mM NaCl and 4μL reservoir solution containing 15% poly(ethylene glycol) 8000, 10% (v/v) isopropanol and 100 mM HEPES (pH 7.4) resulted in crystals of approximate dimensions 0.1 mm × 0.05 mm × 0.05 mm within 4 weeks. Native crystals of jacalin were then soaked in a drop with 50 times molar excess of the ligand in the mother liquor for 48 h to obtain the complexes.

Data Collection, Structure Solution and Validation

X-ray diffraction data from both the complexes were collected at a home source using a MAR345 image plate detector mounted on a Bruker MicroStar rotating anode X-ray generator at 100 K using 25% ethylene glycol as the cryoprotectant. *iMosflm* (15) and *SCALA* (16) in the CCP4 program suite (17) were used to process and scale the intensity data. The intensity data were converted into structure-factor amplitudes using *TRUNCATE* (18) in the CCP4 suite. The data collection statistics along with the cell parameters are given in *Table* 1.

Crystal structures of both the complexes were determined by the molecular-replacement method using *Phaser* (19) from the CCP4 program suite, with the coordinates for native jacalin (PDB Code: 1KU8, (14)) as the initial search model. The solutions obtained from *Phaser* were built using *Coot* v0.7.1 (20) and refined using REFMAC (21). Sugar ligands constructed using *PRODRG* (22) and water O atoms were added to the model when the R and R_{free} were close to 18% and 23%, respectively. The water O atoms were located based on peaks with heights greater than 1σ in $2F_o$-F_c and 3σ in F_o-F_c maps. The possibility of alternate ligand conformations were also evaluated before finalizing the ligand fitting. *PROCHECK* (23) and the *MOLPROBITY* (24) web server were used to validate the refined model. The presence of ligands and their refinement was further confirmed by computing simulated-annealing F_o-F_c *OMIT* maps using *CNS* v.1.3 (25) and contoured at 3σ.

PDB References

Jacalin in complex with GlcNAc β-(1,3) Gal-β-OMe, 5J4T and with Gal β-(1,3) Gal-β-OMe, 5J4X.

Structure Analysis

Alignments of structures for comparison was carried out using ALIGN (26). *PyMOL* (27) was used to generate pictorial representations. *Swiss PDB viewer* (28), *Mercury* (29), *UCSF Chimera* (30) and *Privateer* (31) were used to analyze the structures.

Results and Discussion

Protein–Ligand Interactions

The tertiary structure and quaternary association of jacalin have been thoroughly characterized earlier through X-ray crystallographic studies on several jacalin–sugar complexes (1,4,8,9,11,12,14). The same is true about jacalin–sugar

TABLE 1 ***Data collection and refinement statistics***

Complex	*GlcNAc β-(1,3) Gal-β-OMe (I)*	*Gal β-(1,3) Gal-β-OMe (II)*
Unit cell dimensions		
a (Å)	58.3	58.6
b (Å)	80.7	82.2
c (Å)	62.9	63.1
$\alpha = \gamma$ (°)	90	90
β (°)	107.9	107.4
Resolution (Last shell)	48.85–1.94 (2.04–1.94)	35.71–1.65 (1.74–1.65)
No. of observations	143,681	388,951
No. of unique reflections	40,706	66,777
Completeness (%)	99.1 (94.1)	98.9 (92.8)
$<I/\sigma\ (I)>$	7.9 (4.6)	10.5 (1.9)
R_{merge} (%)[a]	11.1 (22.5)	21.9 (44.3)
Multiplicity	3.5 (3.4)	5.8 (2.8)
R factor (%)	17.55	19.39
R_{free} (%)[b]	22.51	24.03
No. of atoms		
Proteins	4560	4574
Ligands	79	88
Water O atoms	395	555
Rms dev. from ideal values		
Bond lengths (Å)	0.019	0.020
Bond angles (°)	1.952	2.024
Chiral volume	0.127	0.144
Average *B* factors (Å^2)		
Overall	19.0	14.2
Protein	18.0	12.8
Ligands	31.1	30.9
Water O atoms	28.4	22.8
Ramachandran plot		
Core region (%)	88.0	88.2
Additionally allowed region (%)	11.6	11.4
Generously allowed region (%)	0.4	0.4
Disallowed region (%)	0	0
PBD Code	5J4T	5J4X

The crystals belong to space group $P2_1$ and contain a crystallographically independent tetramer.
[a] $R_{merge} = \sum_{hkl}\sum_i|I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$, *where* $I_i(hkl)$ *is the* i*th intensity measurement of a reflection,* $\langle I(hkl)\rangle$ *is the average intensity value of that reflection and the summation is over all measurements.*
[b] *10% of the reflections were used for* R_{free} *calculations.*

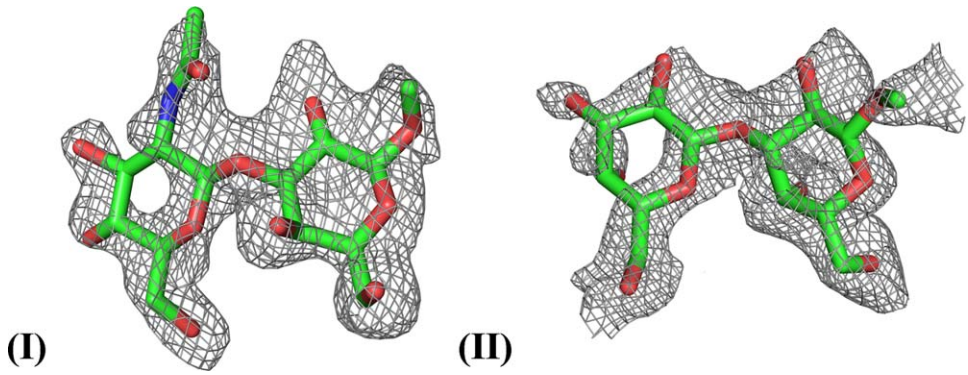

FIG 1

Electron densities in simulated annealing omit maps contoured at 3σ for I and II.

interactions as well. However, significant nuances exist with particular reference to the geometry of the ligand molecules in the binding of the two disaccharides, namely, GlcNAc β-(1,3) Gal-β-OMe (I) and Gal β-(1,3) Gal-β-OMe (II), to jacalin, investigated in this work. The complexes involving (I) and (II) were prepared by soaking the crystals of jacalin (PDB code: 1KU8) (14) in the solutions of the disaccharides. As mentioned earlier (11,12), the binding sites in the four subunits of the molecule in the crystals have different accessibilities and therefore different occupancies of the ligand. In the present case, the ligands are well defined only in subunit-1 (Fig. 1). The protein–ligand interactions and ligand geometries found in this subunit (Fig. 2a,b) are used for further analysis.

The extended binding site of jacalin consists of the primary binding site and secondary binding sites A and B (4,8,12). As in other jacalin–sugar complexes, most of the interactions in the two complexes take place at the primary binding site and involve hydrogen bonds of oxygen atoms in the galactose residue with the side chain of Asp 125, the terminal amino group of the α-chain and the main chain NH/O atoms of Tyr 122 and Trp 123. The side chain of Tyr 78 stacks on the B-face of Gal (1). The hydrophobic pocket involving the side chains of Phe 47, Tyr 78, Tyr 122 and Trp 123 constitute secondary binding site A (4,8). The methyl group of (I) in its complex is in contact with Phe 47 and Tyr 122 while that in the complex involving (II) is in contact with Tyr 78 and Tyr 122. There is no direct protein–sugar interaction at secondary site B. In both the complexes, O6 of the nonreducing sugar forms water bridges with the main-chain atoms of Val 79 and a side chain oxygen atom of Asp 125.

Comparison with Complexes Involving α-Substituted Disaccharides

Available thermodynamic data indicate that β-substituted methyl derivatives of Gal β-(1,3) Gal and related disaccharides bind to jacalin very weakly as compared to the α-substituted methyl derivatives. For instance, the binding constants of Gal β-(1,3) GalNAc-α-OMe and GalNAc β-(1,3) Gal-α-OMe are comparatively high at $1{,}520 \times 10^3$/mol and 490×10^3/mol, respectively (32). The corresponding value for Gal β-(1,3) GalNAc-β-OMe is very low at 0.54×10^3/mol. Structural comparison indicate a similar low value of binding constant for GlcNAc β-(1,3) Gal-β-OMe (I). The binding constant for Gal β-(1,3) Gal-β-OMe (II) has already been shown to be very low at 0.32×10^3/mol (32). The effort here is to provide a structural explanation for the substantial difference in the binding affinities of α-substituted and β-substituted methyl derivatives of β-(1,3) linked disaccharides for jacalin. It is possible to do so, as meaningful interactions of even low affinity ligands to lectins are observed in the crystal structures of lectin–sugar complexes. Documented cases of such interactions of low affinity ligands observed in crystal structures include those of Man and Me-α-Man with *M. smegmatis* lectin (33) and Me-β-Gal with jacalin (11).

The crystal structures of Gal β-(1,3) GalNAc-α-OMe and GalNAc β-(1,3) Gal-α-OMe are available. The binding site and the ligand in the latter, superposed on those involving (I) and (II), are shown in Fig. 2b. The three structures, one involving α-substitution and the other two involving β-substitution, are comparable as they have Gal at the primary binding site. Interactions at the primary binding site are the same in all the complexes. The methyl group nestles in the hydrophobic space at secondary site A in all the three. There is no direct protein–sugar interaction at secondary site B. The water bridges are nearly the same in all the three complexes. Thus, the difference in the binding energy between α- and β-substituted derivatives cannot be explained in terms of protein–sugar interactions.

A close examination revealed that the main difference between the two sets of complexes is in the distortion of the disaccharide in addition to a small lateral shift in the location of the sugars. Distortion of the ligand occurs at two positions. As illustrated in Fig. 3, there is a distortion of the galactose ring at the primary site in the β-substituted disaccharides as compared to the ring in α-substituted disaccharides. The ring conformation in the latter is close to that of Gal in its complex with jacalin (8). When galactose rings are superposed, the plane of C2-C1-O5 in the β-substituted disaccharides deviate by about 5.5° with respect to that in the α-substituted ones.

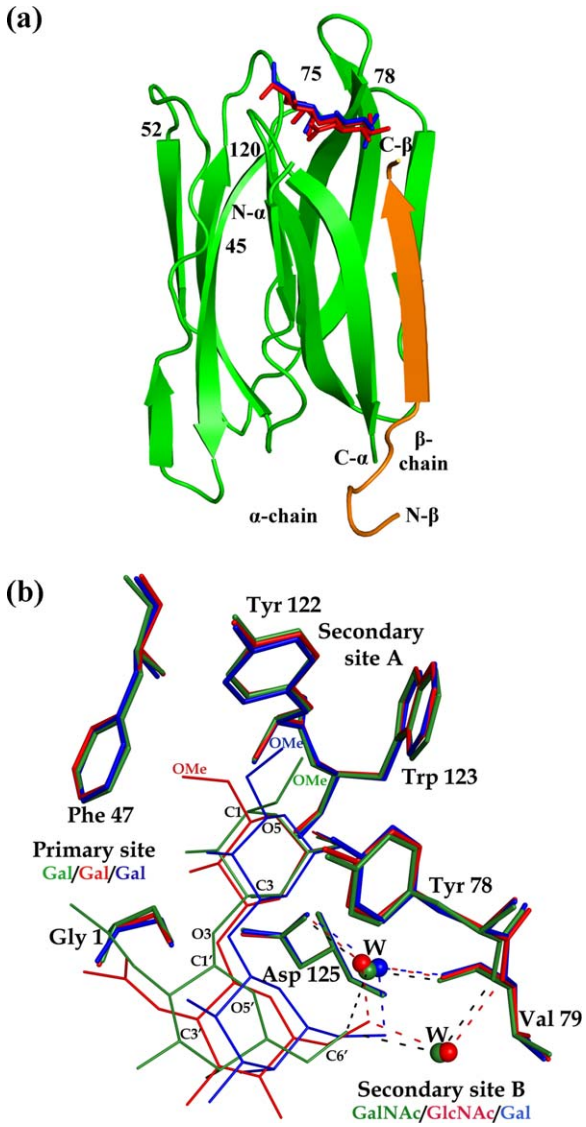

FIG 2

(a) Subunit structure of jacalin. α- and β-chains are indicated in different colors. Locations of the two disaccharides are also shown. Sugar I and sugar II are represented in red and blue, respectively. (b) Locations of I (red), II (blue) and GalNAc β-(1,3) Gal-α-OMe (green) in the respective complexes of jacalin. The binding site residues and the sugar molecules are represented using thick and thin lines respectively. Invariant water molecules involved in water bridges are shown as spheres.

The second distortion is at the glycosidic link. The C3-O3-O1′ angles in the three complexes of jacalin with disaccharides reported earlier (4,8), including two α-substituted methyl derivatives, ranges from 117° to 129°. The ideal value of the angles is 118° (34). The values in (I) and (II) for this angle are 109° and 106°, respectively. These distortions and the slight lateral shifts presumably enable β-substituted β-(1,3) linked disaccharides to have interactions similar to those of the α-substituted derivatives at secondary sites A and B. The higher internal energy of the ligand caused by the distortions is reflected in the lower affinity of the β-substituted disaccharides for jacalin.

It is interesting to compare the present results with those on the binding of β-substituted galactose derivatives to jacalin. There again, the galactose ring gets distorted at C1 to ensure that interactions at secondary site A are similar to those in the complexes involving the corresponding α-substituted derivatives, with a concomitant reduction in the binding affinity (11). The distortion at C1 in the bound β-substituted monosaccharides is however, much larger in terms of the C2-O1-O5 plane than that in the β-substituted disaccharides (Fig. 3). In the disaccharides, the distortion is distributed among C1- and the C3-linked glycosidic linkage, which along with a small lateral shift, appears to ensure the invariance of interactions at secondary sites A and B.

Locational Preference of Gal β-(1,3) Based Disaccharides

The results presented here also led to a re-examination of the locational preference of Gal β-(1,3) Gal based disaccharides when binding to jacalin. Earlier structural studies showed that the disaccharides Gal β-(1,3) GalNAc, Gal β-(1,3) GalNAc-α-OMe and GalNAc β-(1,3) Gal-α-OMe bind to jacalin with the reducing Gal at the primary binding site (4,8). Subsequent modeling studies involving conformational search and energy minimization provided a rationale for the observed locational preference of Gal β-(1,3) Gal (12). The studies showed that an arrangement of Gal β-(1,3) Gal with the reducing sugar at the primary site is preferred over the one with the nonreducing sugar at the site, in terms of accessible conformational space as well as interaction energy. The latter arrangement is also, however, allowed. In the former arrangement, the interaction

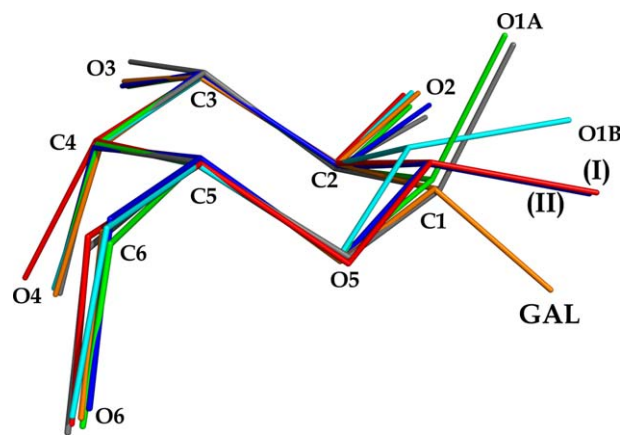

FIG 3

Superposition of the galactose moiety at the primary binding site in complexes involving Gal β-(1,3) GalNAc-α-OMe (gray), GalNAc β-(1,3) Gal α-OMe (green), I (red) and II (blue). Galactose (orange) and methyl β-galactopyranoside (cyan) in their complexes with jacalin are also given for comparison.

energy enhances substantially on α-substitution of a methyl group at the reducing end on account of the interaction of the methyl group at secondary site A. On the contrary, a β-substitution leads to substantial reduction in the interaction energy on account of the distortion of the disaccharide as in the jacalin complexes involving (I) and (II). Simple modeling shows that Gal β-(1,3) Gal-β-OMe can bind to jacalin with the non-reducing Gal at the primary site without any distortion of the ligand, but with no interaction at the secondary site B. However, the crystal structures illustrate that the Gal β-(1,3) Gal-β-OMe still prefers to bind with the reducing Gal at the primary site. Thus, a reduction in the binding affinity on account of the distortion of the ligand is not enough to offset the intrinsic advantage of the location of the disaccharide with the reducing end at the primary binding site.

Conclusions

Interactions of derivatives of Gal β-(1,3) Gal-α-OMe with jacalin have been structurally and thermodynamically well characterized. It is now shown that β-substituted methyl derivatives of the disaccharides can also bind to jacalin without changing the pattern of interactions in the complexes involving the corresponding α-substituted derivatives. This is achieved through distortions of the ligand molecule at the anomeric carbon and the glycosidic linkage in addition to a small lateral shift. The higher internal energy caused by the distortion is reflected in the lower affinity of β-substituted β-(1,3) linked disaccharides to jacalin as compared to that of the α-substituted variants. The present work thus confirms distortion of the ligand as a strategy for modulating affinity.

It has been demonstrated earlier that Gal β-(1,3) Gal and its derivatives prefer to bind to jacalin with the reducing sugar at the primary binding site, although binding with the nonreducing Gal at the primary site is possible. α-Methyl substitution further strengthens binding in the first arrangement. β-Substitution, on the contrary, weakens the binding on account of the distortion of the ligand. The β-substituted disaccharides still continue to bind with the reducing Gal at the primary binding site indicating that the reduction of affinity is not strong enough to overcome the intrinsic propensity of Gal β-(1,3) Gal to bind to jacalin with the reducing Gal at the primary site. This propensity is indeed an important determinant in the biologically relevant interactions of jacalin with oligosaccharides.

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