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Jacalin–carbohydrate interactions: distortion of the ligand molecule as a determinant of affinity

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Jacalin is among the most thoroughly studied lectins. Its carbohydrate-binding site has also been well characterized. It has been postulated that the lower affinity of β-galactosides for jacalin compared with α-galactosides is caused by steric interactions of the substituents in the former with the protein. This issue has been explored energetically and structurally using different appropriate carbohydrate complexes of jacalin. It turns out that the earlier postulation is not correct. The interactions of the substituent with the binding site remain essentially the same irrespective of the anomeric nature of the substitution. This is achieved through a distortion of the sugar ring in β-galactosides. The difference in energy, and therefore in affinity, is caused by a distortion of the sugar ring in β-galactosides. The elucidation of this unprecedented distortion of the ligand as a strategy for modulating affinity is of general interest. The crystal structures also provide a rationale for the relative affinities of the different carbohydrate ligands for jacalin.

1. Introduction

Lectins, which are described as multivalent carbohydrate-binding proteins, were first discovered in plants and their best known property is their ability to agglutinate red blood cells. They therefore used to be described as phytohaemagglutinins (Rüdiger & Gabius, 2001; Hamblin & Kent, 1973). They were subsequently found in all kingdoms of life, including bacteria and viruses (Chandra *et al.*, 2006). They are involved in a plethora of biological processes, including cell–cell interactions, malignancy, cellular signalling, differentiation and immune response (Sharon & Lis, 1989; Lis & Sharon, 1998; Drickamer, 1999; Rini & Lobsanov, 1999; Vijayan & Chandra, 1999). Lectins exhibit a variety of folds and quaternary associations. The only thing common to them is their ability to specifically bind different carbohydrate structures. Plant lectins of known three-dimensional structure mostly belong to five major classes (http://www.cermav.cnrs.fr/lectines), including one discovered in our laboratory through the X-ray analysis of jacalin (Sankaranarayanan *et al.*, 1996), which is one of the two lectins found in jackfruit seeds (*Artocarpus integrifolia*). Our efforts on plant lectins have encompassed three more of these classes and have yielded results of general interest in relation to quaternary association, strategies for generating ligand specificity *etc.*, in addition to those pertaining to specific lectins (Banerjee *et al.*, 1994; Sankaranarayanan *et al.*, 1996; Chandra *et al.*, 1999; Natchiar *et al.*, 2007; Sharma *et al.*, 2013; Chandran *et al.*, 2013).

Jacalin is a tetrameric glycosylated protein with a molecular weight of 66 kDa which is galactose-specific at the monosaccharide level (Sankaranarayanan *et al.*, 1996). Each subunit of jacalin is made up of two chains (Young *et al.*, 1991; Mahanta *et al.*, 1992; Sankaranarayanan *et al.*, 1996) generated by post-translational proteolysis (Yang & Czapla, 1993). The shorter N-terminal fragment is termed the β-chain and the longer C-terminal fragment is called the α-chain. The N-terminus of the α-chain is important for generating specificity for galactose, thus establishing post-translational proteolysis as a strategy for generating ligand specificity (Sankaranarayanan *et al.*, 1996). At the disaccharide level, jacalin has high affinity for the tumour-associated T-antigenic Galβ(1–3)-GalNAc (Sastry *et al.*, 1986). Crystal structure analyses and thermodynamic studies on the interactions of jacalin with several galactose derivatives have been carried out (Mahanta *et al.*, 1990; Sankaranarayanan *et al.*, 1996; Jeyaprakash *et al.*, 2002, 2003, 2005). These have led to a detailed understanding of the extended binding site of jacalin. A secondary region of the site is of great importance as it facilitates the weak binding of methyl α-mannose (Me-α-Man) to the lectin primarily through the interaction of the methyl group with this region (Bourne *et al.*, 2002; Jeyaprakash *et al.*, 2005). Further insights into jacalin–sugar interactions, particularly in relation to conformational selection and induced fit, have been gained through extensive molecular-dynamics simulations (Sharma *et al.*, 2009).

The second lectin from jackfruit seeds, artocarpin, is also tetrameric and has a β-prism I fold, but it is nonglycosylated and mannose-specific, with a single-chain subunit. The structure and interactions of artocarpin have also been thoroughly elucidated in our laboratory (Pratap *et al.*, 2002; Jeyaprakash *et al.*, 2004). Structure analyses of many other β-prism I fold lectins and their sugar complexes have been carried out in our laboratory and elsewhere (Chandran *et al.*, 2013). Their sequences, evolutionary history and quaternary association have also been explored (Sharma *et al.*, 2007; Sharma & Vijayan, 2011). However, even in the case of jacalin, the most thoroughly studied β-prism I fold lectin, some unanswered questions still remain. One of them concerns the structural basis for the differential affinity of α-and β-substituted sugars for jacalin. This issue is specifically explored here using complexes of jacalin with α-substituted as well as β-substituted galactose derivatives involving methyl, *p*-nitrophenyl and 4-methylumbelliferyl groups. Contrary to expectation, the lower affinity of the β-substituted derivatives appears to be caused by distortion of the sugar molecules and not by steric clashes involving the substituent, which is a result of considerable general interest.

2. Materials and methods

2.1. Isothermal calorimetric measurements

2.1.1. Preparation of solutions. Jacalin was extracted from crude jackfruit seeds by affinity purification by passing the crude seed extract through a galactose cross-linked guar gum column and eluting it with galactose in phosphate-buffered saline (Kumar *et al.*, 1982). The purified protein was further dialyzed against 20 m*M* phosphate buffer pH 7.3, 150 m*M* NaCl, 0.025% sodium azide to remove all of the galactose bound to the protein. Haemagglutination was measured after the purification of every batch to ascertain the activity of the purified batch. Protein concentrations were checked using spectrophotometry. A solution of nonfluorescent sugar and the protein was prepared by dialysis to maintain the homogeneity of the buffer of the protein and ligand. The concentrations of nonfluorescent sugars were measured using the modified DuBois phenol/chloroform method (DuBois *et al.*, 1956), while for fluorescent sugars concentrations were measured spectrophotometrically at 318 nm.

2.1.2. Experimental setup for ITC. All of the titrations were conducted using a MicroCal VP-ITC calorimeter at 273, 283, 288, 293 and 298 K. The total concentration M_t of jacalin that was used in the titrations varied from 1 to 4 m*M* (of subunit), while the total concentration X_t of nonfluorescent sugar used was in the range 12–80 m*M* for the titrations with galactose (Gal), methyl α-galactose (Me-α-Gal) and methyl β-galactose (Me-β-Gal). For the fluorescent sugars, namely *p*-nitrophenyl α-galactose (PNP-α-Gal), *p*-nitrophenyl β-galactose (PNP-β-Gal), 4-methylumbelliferyl α-galactose (MUF-α-Gal) and 4-methylumbelliferyl β-galactose (MUF-β-Gal), the concentration used for the titration was in the range 2000–3500 μM. The optimal *C*-value in ITC calculation varied between 0.375 and 129 ($n \times K_b \times M$, where n is the number of binding sites, K_b is the binding constant and M is the protein concentration). 1.36 ml lectin solution was added to the sample cell, ensuring that there were no trapped air bubbles, and the mixture was equilibrated at different temperatures. Sugar solutions were added as a series of injections (35 aliquots of 5–7 µl) at 3 min intervals by means of a computer-controlled syringe (250 µl) with constant stirring at 396 rev min^{-1}. The spacing between two injections helped to achieve a steady baseline of titration. Titrations were performed with ligand and buffer to confirm that the heat generated by the ligand was negligible. Data were analyzed using *OriginPro* 7.0 (OriginLab) and fitted using the 'single set of sites' model incorporated into the software. Measurements of the heat change were determined from the binding constant (K_b), reaction stoichiometry (n) and enthalpy (ΔH). Free energy (ΔG) and binding entropy (ΔS) were calculated by the equations $\Delta G = -RT\ln K_b$ and $\Delta G = \Delta H - T\Delta S$, where R is the gas constant and T is the absolute temperature. The n values were approximated as 1 in all of the calculations.

2.2. Crystallization and data collection

2.2.1. Crystallization. Native crystals of jacalin were grown in conditions similar to those described in Bourne *et al.* (2002) from a 12 mg ml^{-1} protein solution using 15% polyethylene glycol 8000, 10%(*v*/*v*) 2-propanol in 0.1 *M* HEPES pH 7.4 as the precipitant by the vapour-diffusion technique at 25°C. Drops containing equal volumes of protein solution and precipitant solution were equilibrated against the precipitant

Table 1
Data-collection and refinement statistics for the jacalin–ligand complexes.

Values in parentheses are for the last shell.

Complex/PDB code	Me-β-Gal/4r6n	PNP-α-Gal/4r6q	PNP-β-Gal/4r6r	MUF-α-Gal/4r6o	MUF-β-Gal/4r6p
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Soaking time	10 min	48 h	48 h	1 h	24 h
Concentration	40 m*M*	10 m*M*	500 µ*M*	10 m*M*	350 µ*M*
No. of bound sites	3	4	3	4	1
Unit-cell parameters					
a (Å)	58.53	58.63	58.44	58.59	58.53
b (Å)	80.90	81.96	80.25	82.18	80.88
c (Å)	63.21	63.31	62.97	63.31	63.04
$\alpha = \gamma$ (°)	90	90	90	90	90
β (°)	108.08	107.12	107.68	106.76	107.88
Resolution (Å)	45.96–1.68 (1.77–1.68)	28.02–1.60 (1.69–1.60)	24.44–1.38 (1.46–1.38)	28.05–1.55 (1.63–1.55)	30.63–1.70 (1.79–1.70)
No. of observations	353581	252597	467511	345997	324752
No. of unique reflections	62327	72829	113064	83181	60267
Completeness (%)	97.1 (92.3)	96.7 (95.3)	99.8 (99.0)	99.9 (100)	98.2 (97.1)
$\langle I/\sigma(I)\rangle$	10.8 (2.3)	6.5 (1.9)	10.1 (1.7)	8.2 (1.9)	11.5 (2.1)
$R_{\rm merge}$ (%)†	14.3 (31.3)	9.5 (50.8)	6.5 (82.4)	8.9 (55.9)	7.7 (76.1)
Multiplicity	5.7 (1.8)	3.5 (3.5)	4.1 (3.8)	4.2 (4.1)	5.4 (5.4)
R factor/$R_{\rm free}$‡ (%)	17.02/22.09	18.44/22.86	17.77/20.67	18.09/21.88	16.60/21.13
No. of atoms					
Proteins	4608	4547	4579	4567	4552
Ligands	131	116	98	148	44
Water O atoms	628	603	437	673	475
R.m.s. deviations from ideal values					
Bond lengths (Å)	0.020	0.020	0.019	0.019	0.011
Bond angles (°)	2.038	2.072	1.987	2.071	1.475
Chiral volume (Å^3)	0.148	0.141	0.148	0.148	0.106
Wilson plot *B* factor (Å^2)	11.2	18.7	16.0	20.2	21.5
Average *B* factors (Å^2)					
Overall	14.4	21.3	21.1	22.1	26.9
Protein	12.0	19.1	19.6	19.8	25.3
Ligands	24.7	30.5	38.5	34.0	50.4
Water O atoms	29.6	36.6	32.2	35.2	40.7
Ramachandran plot					
Core region (%)	88.4	89.6	89.9	88.8	89.9
Additionally allowed region (%)	11.6	10.4	9.9	10.8	10.1
Generously allowed region (%)	0	0	0.2	0.4	0
Disallowed region (%)	0	0	0	0	0

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, 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. ‡ 5% of the reflections were used for the $R_{\rm free}$ calculations.

solution. Good-quality crystals of jacalin with approximate dimensions of 0.1 × 0.05 × 0.05 mm were obtained in four weeks. Since co-crystallization attempts using all of the ligands were not successful, soaking experiments were conducted to obtain the crystals of jacalin–ligand complexes.

2.2.2. Data collection and processing. Data from the crystals of the MUF-α-Gal, MUF-β-Gal, PNP-α-Gal and PNP-β-Gal complexes were collected on the BM-14 beamline at a wavelength of 0.98 Å at the European Synchrotron Radiation Facility (ESRF), Grenoble, France using a MAR225 CCD. Data from the Me-β-Gal complex were collected at the home source using a MAR345 image-plate detector mounted on a Bruker MicroStar rotating-anode X-ray generator. All data sets were collected at 100 K using 25% ethylene glycol as the cryoprotectant. The collected intensity data were processed and merged using *iMosflm* (Battye *et al.*, 2011) and scaled with *SCALA* in the *CCP*4 program suite (Winn *et al.*, 2011). The intensity data were converted into structure-factor amplitudes using *TRUNCATE* (French & Wilson, 1978) from the *CCP*4 suite. The data-collection statistics along with the unit-cell parameters are given in Table 1.

2.3. Structure refinement and validation

The structures were refined using *REFMAC* (Murshudov *et al.*, 2011) from *CCP*4 and model building was carried out using *Coot* v.0.7.1 (Emsley & Cowtan, 2004) with the coordinates provided in PDB for native jacalin (PDB entry 1ku8) as the starting model. Addition of sugar ligands and water O atoms took place using *PRODRG* (Schüttelkopf & van Aalten, 2004) when *R* and $R_{\rm free}$ were close to 22 and 27%, respectively. The water O atoms were located based on peaks with heights greater than 1.0σ in $2F_{\rm o} - F_{\rm c}$ maps and 3σ in $F_{\rm o} - F_{\rm c}$ maps. The refined models were validated using *PROCHECK* (Laskowski *et al.*, 1993) and the *MolProbity* (Chen *et al.*, 2010) web server. Refinement statistics are also summarized in Table 1.

2.4. Analyses and modelling

Structure alignments were carried out using *ALIGN* (Cohen, 1997). All pictorial illustrations were generated using *PyMOL* (http://www.pymol.org).

Table 2
Isothermal calorimetric data for Gal and its derivatives at 298 K.

The ΔC_p values were calculated using the temperature-dependent data given in Supplementary Table S1.

Complex	$K_b \times 10^{-3}$ (M^{-1})	$-\Delta H_b$ (kJ mol^{-1})	$-\Delta G_b$ (kJ mol^{-1})	$-T\Delta S$ (kJ mol^{-1})	ΔC_p (kJ mol^{-1} K^{-1})
D-Gal (Gal)	0.80 (± 0.03)	23.17 (± 0.09)	16.56	6.57	0.029
Methyl α-D-Gal (Me-α-Gal)	20.0 (± 0.21)	46.88 (± 0.08)	24.53	22.33	0.267
Methyl β-D-Gal (Me-β-Gal)	0.15 (± 0.003)	11.51 (± 0.14)	12.41	−0.92	0.199
p-Nitrophenyl α-D-Gal (PNP-α-Gal)	77.9 (± 1.10)	49.39 (± 0.07)	27.91	21.46	0.481
p-Nitrophenyl β-D-Gal (PNP-β-Gal)	2.37 (± 0.08)	38.76 (± 1.12)	19.23	19.46	0.515
UMB α-D-Gal (MUF-α-Gal)	335 (± 15.10)	70.19 (± 0.25)	31.52	38.67	0.785
UMB β-D-Gal (MUF-β-Gal)	12.9 (± 0.86)	33.04 (± 0.78)	23.45	9.57	0.542

3. Results and discussion

3.1. Thermodynamic parameters

Thermodynamic parameters for the interaction of all of the sugars with jacalin were measured at five temperatures using isothermal titration calorimetry (ITC; Table 2, Supplementary Table S1). The relative trends in the interaction of the different sugars are the same at the five temperatures. The number of binding sites is one per monomer in all cases. The affinity of Me-β-Gal is lower than that of galactose (Gal), while that of Me-α-Gal is higher. The affinities of the *p*-nitrophenyl (PNP) and 4-methylumbelliferyl (MUF) derivatives are higher than that of Gal irrespective of the anomeric nature of the substituent. PNP-α-Gal and MUF-α-Gal have a much greater affinity than Me-α-Gal. The same is true in relation to the corresponding β-substituents. MUF-α-Gal has a higher affinity for jacalin than PNP-α-Gal. Likewise, MUF-β-Gal has a higher affinity than PNP-β-Gal. As expected, the α-substituted sugar binds to the protein with a substantially higher affinity than the β-substituted sugars in all three cases. The variation in ΔC_p among the complexes also indicates stronger nonpolar interactions of the bulkier ligands with the lectin.

3.2. Molecular structure and the carbohydrate-binding site

Only one uncomplexed crystal structure of jacalin is available to date (Bourne *et al.*, 2002). Crystals of this native form were used to prepare, by soaking, the five complex crystals for which structures are reported here. The uncomplexed molecule and the molecules complexed with sugars presented here and reported previously (Sankaranarayanan *et al.*, 1996; Jeyaprakash *et al.*, 2002, 2003, 2005) have the same tertiary and quaternary structures (Fig. 1). The tertiary structure involves three Greek keys arranged as three sides of a nearly threefold-symmetric prism. One of them has a break on account of the post-translational proteolysis referred to earlier. The N-terminus thus generated is close to the sugar-binding loops, which are located at one end of the prism. The subunits arrange themselves into a tetramer with 222 symmetry.

Loops 46–52, 76–82 and 122–125, along with the N-terminus (Gly1) of the α-chain generated by post-translational proteolysis, constitute the binding site in each subunit. A composite view of the residues involved in the interactions with sugars in the native structure, in the five complexes presented here and in the original structure of the complex with Me-α-Gal (Sankaranarayanan *et al.*, 1996) is given in Fig. 2. The side chains of Phe47, Tyr78 and Asp125, the backbone N and O atoms of Tyr122 and Trp123 and the free amino-terminus of the α-chain constitute the primary binding site. Of these, the side chain of Tyr78 stacks against the galactose ring in the complexes. The secondary binding site A is composed of the aromatic side chains of Tyr78, Tyr122 and Trp123. The backbone N and O atoms of Val79, Ser119 OG and the carboxyl-terminal region of the β-chain make up the secondary binding site B. This site makes no direct interactions with the bound sugars. They are involved in water bridges in some of the complexes.

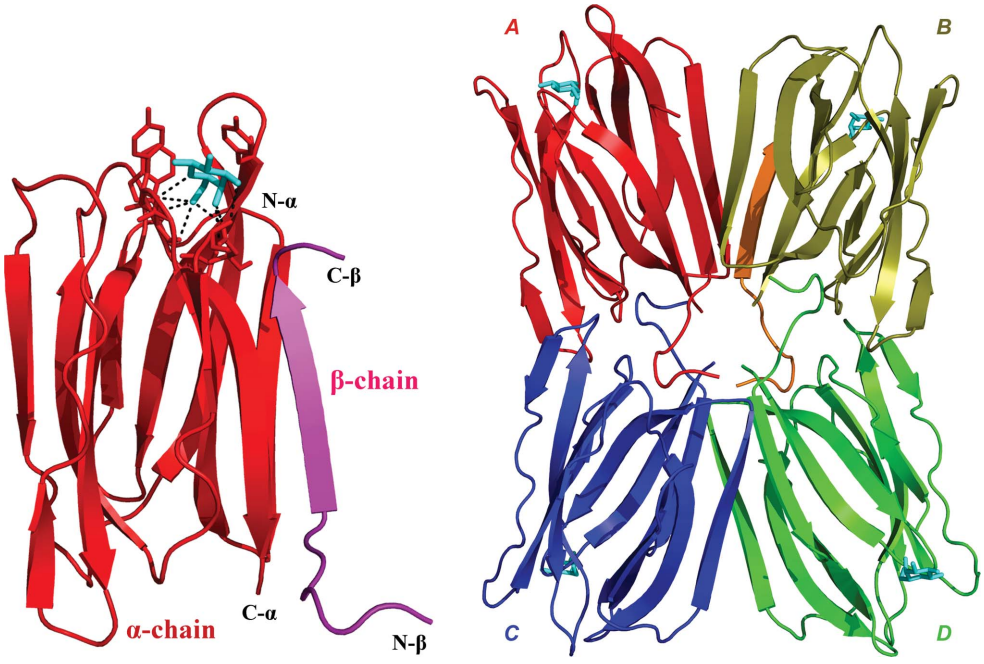

Figure 1
Structures of a subunit of jacalin and the tetrameric molecule.

3.3. Conformational selection and induced fit

The binding sites in plant lectins are believed to be

Table 3
Occupation of binding sites in the complexes.

Site No.	Notation	Subunit *A*	Subunit *B*	Subunit *C*	Subunit *D*
1	Me-β-Gal	Yes	No	Yes	Yes
2	PNP-α-Gal	Yes	Yes	Yes	Yes
3	PNP-β-Gal	Partially defined	No	Yes	Yes
4	MUF-α-Gal	Yes	Yes	Yes	Yes
5	MUF-β-Gal	No	No	No	Partially defined

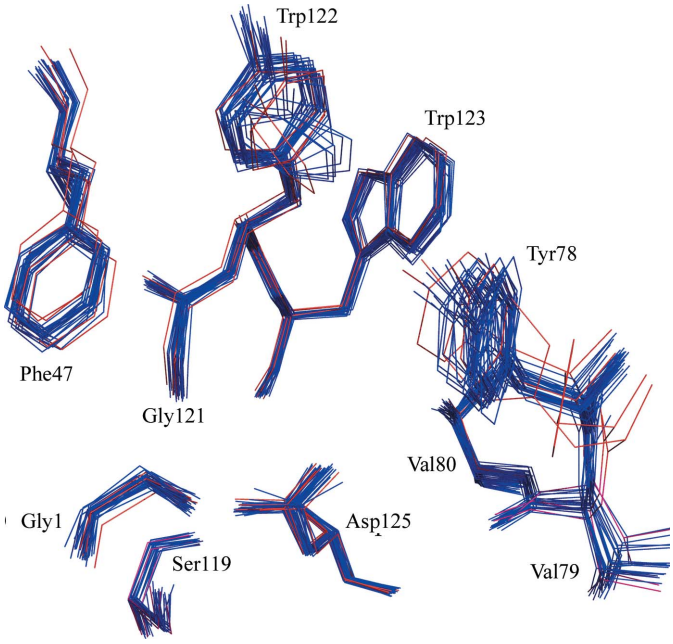

Figure 2
Superimposition of the binding sites of jacalin in the native structure (red) and the relevant complexes (blue). See text for details.

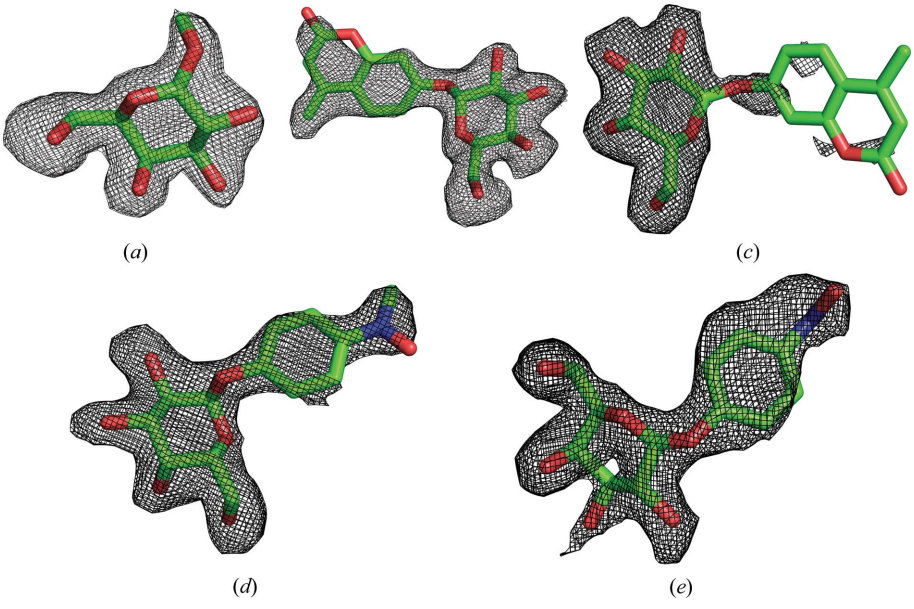

Figure 3
Typical electron densities in $F_o - F_c$ maps for ligands in different complexes: (*a*) Me-β-Gal in subunit *A*, (*b*) MUF-α-Gal in subunit *C*, (*c*) MUF-β-Gal in subunit *D*, (*d*) PNP-α-Gal in subunit *D* and (*e*) PNP-β-Gal in subunit *D*.

substantially preformed. This is also true for jacalin. However, flexibility is seen within the comparatively rigid framework. The flexibility is particularly evident for the aromatic residues Phe47, Tyr78 and Tyr122. Interestingly, these residues exhibit substantial structural variability in the four subunits of the native jacalin structure (Fig. 2). In the native structure in the absence of bound sugar, Tyr78, which stacks against the galactose ring, is free to move. The side chain of Tyr122 interacts with the groups attached to the anomeric O atom in the complexes. Again, it is free to move in the absence of bound sugar. However, it is interesting to note that the locations of these two residues in the complexes are largely within the range exhibited in the native structure (Fig. 2), perhaps exemplifying a case of conformational selection. Some locations are outside this range. Thus, the real situation involves a combination of conformational selection and induced fit.

3.4. Structures of complexes involving α- and β-substituted galactose derivatives

The low affinity of Me-β-Gal compared with that of Me-α-Gal has been suggested to be caused by steric clashes of the methyl group in the former with secondary site A (Jeyaprakash *et al.*, 2002, 2003; Sharma & Vijayan, 2011; Chandran *et al.*, 2013). This suggestion was not, however, explored using X-ray structural studies. Furthermore, the higher affinity of PNP-β-Gal and MUF-β-Gal appeared to be inconsistent with the hypothesis involving steric clashes. PNP and MUF are bulkier than the methyl group and therefore steric clashes would be expected to be more severe in the case of PNP-β-Gal and MUF-β-Gal. Thus, it appeared that this issue merited further investigation, and this was the motivation for the present structural work.

The packing environments of the binding sites in the four subunits are not the same in the crystal form used in the present work. In particular, access to the binding site in subunit *B* is restricted on account of the close approach of Tyr122 from subunit *D* of a 2_1 screw-related neighbouring molecule. Consequently, as can be seen from Table 3, the binding site of subunit *B* is not occupied in the majority of the structures. It is only occupied when the ligand is PNP-α-Gal or MUF-α-Gal. Presumably, the affinities of these two ligands are strong enough to push the neighbouring molecule away to achieve binding. This interpretation appears to be corroborated by the longer *b* cell dimension in the crystals of the two complexes (Table 1). The close approach of the subunits in the two molecules has the additional consequence of the second residue of the MUF-α-Gal complex in its complex interacting extensively with

Table 4
Deviation (°) of the C2—C1—O5 plane from that in subunit *A* of the Gal complex.

Gal derivative	Deviation (°)
Gal	
A	0
B	1.7
C	0.8
D	1.9
Me-α-Gal	
A	1.3
B	1.1
C	3.0
D	3.9
Me-β-Gal	
A	16.7
C	11.1
D	14.6
PNP-α-Gal	
A	2.0
B	2.0
C	3.0
D	2.1
PNP-β-Gal	
C	9.6
D	5.5
MUF-α-Gal	
A	0.4
B	2.5
C	1.3
D	6.2

subunit *B* of the neighbouring molecule. It is not entirely clear why the sites in subunits *A* and *C* are unoccupied in the MUF-β-Gal complex.

The ligands have well defined electron density at the occupied sites except in the case of PNP-β-Gal at subunit *A* and the lone site occupied by MUF-β-Gal in subunit *D* (Fig. 3). Density exists for the galactose moiety at these two sites. This means that the geometry of MUF in MUF-β-Gal is not well defined in any structure. Thus, jacalin–sugar interactions are well defined in three subunits for Me-β-Gal, two subunits for PNP-β-Gal and four subunits each for PNP-α-Gal and MUF-α-Gal. These and the well defined interactions in four subunits for Gal (Jeyaprakash *et al.*, 2003) and Me-α-Gal (Sankaranarayanan *et al.*, 1996) form the basis for the analysis that follows.

3.5. Distortion of the sugar ring

To start with, the possibility that the Gal moiety of the β-substituted derivatives could have a different orientation in their complexes compared with that in α-substituted derivatives was explored. However, lectin–sugar interactions involving the galactose moiety, as illustrated in Fig. 4, are the same in the two sets of complexes. Furthermore, no serious steric clashes involving secondary binding site A and the sugar could be observed in complexes involving the β-substituted derivatives. However, models involving the β-substituted sugars generated by switching the anomeric O atom in the complexes of α-substituted sugars led to steric clashes. A closer examination then revealed that the sugar ring in the β-substituted derivatives is distorted in their complexes with respect to the geometry in the α-substituted derivatives.

A superposition of the galactose moiety in the complexes involving Gal and Me-α-Gal and the structures reported here is shown in Fig. 5. In the Gal complex, the β-anomer is well defined in all four subunits and C1 and O1 cluster together. C1 in the α-substituted derivatives clusters together with that in Gal. O1 in all of them clusters together separately. Interestingly, in the complexes involving β-substituted sugars the ring is distorted and C1 forms a separate cluster. O1 in these derivatives clusters together separately. Thus, in the jacalin complexes, the ring conformation in the α-substituted sugars is the same as that in Gal. The ring is distorted in the β-substituted sugars.

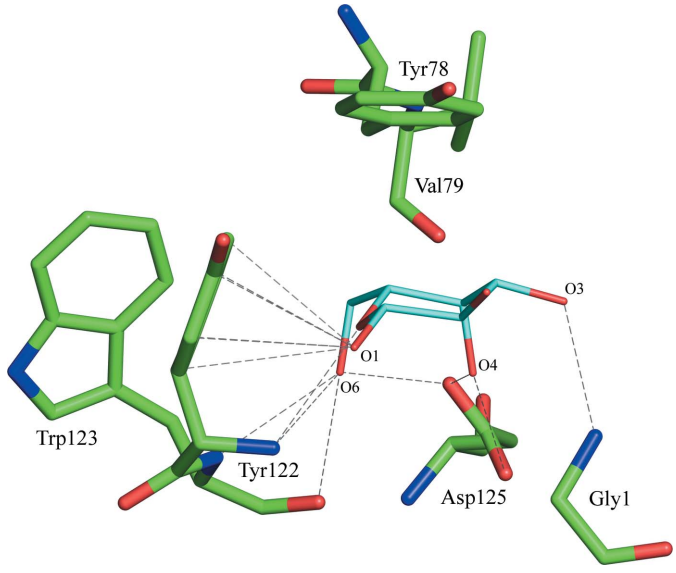

Figure 4
Interaction of β-Gal with jacalin in subunit *D* of the Gal complex.

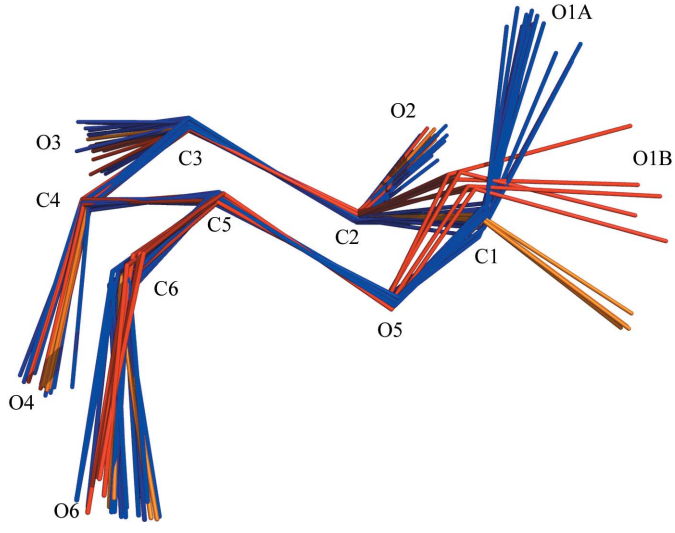

Figure 5
Superposition of the galactose moiety in complexes involving Gal (orange), α-substituted sugars (blue) and β-substituted sugars (red).

In all of the structures considered, all of the atoms except for the ring atom C1 in the galactose moiety superpose reasonably well. One way of describing the deviation of C1 among the sugar molecules is in terms of the deviation of the C2—C1—O5 plane (Table 4). Taking the plane in the ligand molecule in subunit *A* of the jacalin–galactose complex as a reference, the angle between the planes in the Gal complex varies between 0 and 1.9°. The range for the α-substituted sugars in their complexes, except in subunit *D* of the MUF-α-Gal complex, is 0.4–3.9°, which is comparable to the range in the Gal complex. The anomalously high value exhibited by the ligand in subunit *D* of the MUF-α-Gal complex is presumably on account of the extensive interactions of the ligand with subunit *B* of a neighbouring molecule referred to earlier. The values in the β-substituted derivatives in complex with jacalin are higher and range from 9.6 to 16.7°. The distortion arising from the movement of C1 naturally involves distortion in two bond angles, C1—C2—C3 and C1—O5—C5. The average values of the former in the complexes involving α-substituted sugars are 111°, a value close to the tetrahedral angle. The corresponding value in those involving β-substituted sugars is lower at 102°. The average values of C1—O5—C5 in the two cases are 113 and 102°, respectively.

3.6. Interactions at the secondary binding site A

Despite the substantial difference in the orientation of the anomeric O atom in the α-substituted and β-substituted derivatives, the orientations of substituents with respect to the binding site are very similar in the two sets of complexes (Fig. 6). In the complexes involving the methyl derivative, the methyl group interacts with the aromatic side chain of Tyr122. The existence of a C—H$\cdots\pi$ hydrogen bond is determined on the basis of the distance between the methyl C atom and the centre of the aromatic ring (*M* in Å) and the angle between the line joining the two and normal to the aromatic plane (ω in degrees). A C—H$\cdots\pi$ hydrogen bond is taken to exist if *M* is less than 3.5 Å and ω is less than 25° (Steiner & Koellner, 2001). *M* and ω range from 3.2 to 3.9 Å and from 8 to 18°, respectively, in Me-α-Gal bound to jacalin. The corresponding values for Me-β-Gal are 3.4–3.5 Å and 4–8°, respectively. Thus, the strengths of interaction involving the methyl group are comparable in the two cases.

The phenyl ring in PNP-α-Gal and PNP-β-Gal stacks against the side chain of Tyr122 in their respective complexes. A stacking interaction is defined by the distance between the centres of the two rings (*N* in Å) and the angle between the two planar groups (θ in degrees) (Hunter *et al.*, 1991). *N* varies between 3.5 and 4.3 Å and θ varies between 6 and 24° in the complexes involving α-substituents. In the two subunits in which PNP-β-Gal is fully defined, *N* is 3.6 Å in both cases and θ varies from 7 to 12°. Again, the strengths of the two sets of interactions are comparable. A similar comparison is not possible in the case of MUF-Gal, as MUF-β-Gal is not fully defined in any of the subunits in the complex. Thus, irrespective of the anomeric nature of the substitution the substituents interact in a similar manner with the secondary binding site *A*, particularly with Tyr122. This is achieved primarily by modulation of the orientation of the anomeric O atom in β-substituted derivatives consequent to the distortion of the sugar ring.

3.7. Distortion of the ligand as a modulator of affinity

Isothermal calorimetric data clearly show that the free energy and the enthalpy of complex formation with a given sugar are always significantly lower for an α-substituted derivative compared with those for the corresponding β-substituted derivative. For a given anomeric substitution, bulkier substituents yield better (lower) free energies and enthalpies. This is presumably on account of the more extensive interactions of the bulkier substituents with the secondary binding site A.

4. Conclusions

The difference between the free energy of a binary complex and the sum of the free energies of the individual components determines the affinity between the components. As far as the enthalpy component is concerned, the interactions between the two components determines the affinities if the energies of the components do not change during complexation. In the present structures, the interaction between a given derivative and the lectin remain the same, irrespective of the anomeric

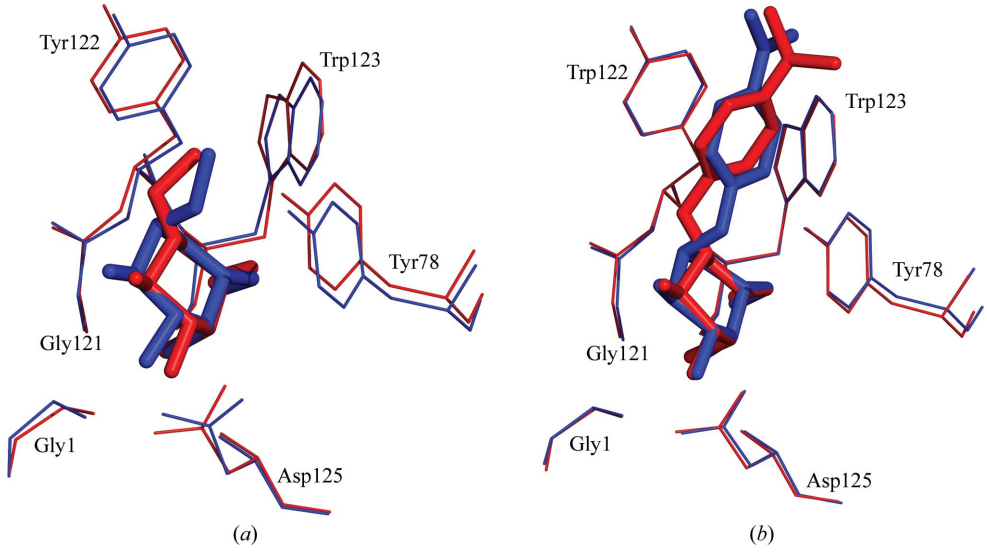

Figure 6
(*a*) Me-α-Gal (blue) and Me-β-Gal (red) and (*b*) PNP-α-Gal (blue) and PNP-β-Gal (red) in the carbohydrate-binding site of subunit *D* in jacalin.

nature of the substitution. However, the ring and the two bond angles become distorted, presumably leading to higher internal energy of the ligand in the case of β-substituted derivatives. This appears to be the reason for the apparent lower affinity of the β-substituted derivatives compared with the corresponding α-substituted derivatives. The results presented here thus show, perhaps for the first time, how distortion of the ligand molecule can be used to modulate affinity.

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