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Porphyrin binding to jacalin is facilitated by the inherent plasticity of the carbohydrate-binding site: novel mode of lectin–ligand interaction

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The crystal structure of the complex of *meso*-tetrasulfonatophenylporphyrin (H_2TPPS) with jack fruit (*Artocarpus integriflora*) agglutinin (jacalin) has been determined at 1.8 Å resolution. A porphyrin pair is sandwiched between two symmetry-related jacalin monomers in the crystal, leading to a cross-linking network of protein molecules. Apart from the stacking interactions, H_2TPPS also forms hydrogen bonds, some involving water bridges, with jacalin at the carbohydrate-binding site. The residues that are involved in rendering galactopyranoside specificity to jacalin undergo conformational adjustments in order to accommodate the H_2TPPS molecule. The water molecules at the carbohydrate-binding site of jacalin cement the jacalin–porphyrin interactions, optimizing their complementarity. Interactions of porphyrin with jacalin are relatively weak compared with those observed between galactopyranoside and jacalin, perhaps because the former largely involves water-mediated hydrogen bonds. While H_2TPPS binds to jacalin at the carbohydrate-binding site as in the case of ConA, its mode of interaction with jacalin is very different. H_2TPPS does not enter the carbohydrate-binding cavity of jacalin. Instead, it sits over the binding site. While the porphyrin binding is mediated by replicating the hydrogen-bonding network of mannopyranoside through the sulfonate atoms in the case of ConA, the plasticity associated with the carbohydrate-binding site accommodates the pluripotent porphyrin molecule in the case of jacalin through an entirely different set of interactions.

1. Introduction

The structural basis of the specificity associated with molecular recognition involving proteins and their ligands, crucial for understanding cellular mechanisms at the molecular level, has been a complex problem. Although it has generally been accepted that molecular recognition involves complementarity of shape and charge at the interface, it is becoming increasingly evident that other structural properties also contribute to conferring specificity. Therefore, molecular mimicry (functional equivalence of the chemically independent molecules) may not necessarily imply structural correlation. In other words, the specificity of molecular recognition need not be evident through obvious structural characteristics. Therefore, in order to delineate the structural properties that distinguish molecular mimicry and specificity of recognition, it is imperative to systematically analyze molecular interactions involving receptor-ligand systems. In this context, we have been investigating diverse lectin–ligand interactions at the structural as well as the physiological level (Kaur *et al.*, 1997, 2001; Jain, Kaur, Goel *et al.*, 2000; Jain, Kaur, Sundaravadivel *et al.*, 2000; Jain *et al.*, 2001*a*,*b*; Goel *et al.*, 2001).

Important insights have emerged with regard to peptide–carbohydrate mimicry as manifested in the humoral immune response from our structural and immunological investigations involving mannopyranoside and its dodecapeptide mimic (Kaur *et al.*, 1997; Jain, Kaur, Sundaravadivel *et al.*, 2000). Analysis of polyclonal antibody response involving mannopyranoside and the dodecapeptide provided topological correlation between the carbohydrate moiety and the YPY motif from the peptide molecule (Jain, Kaur, Goel *et al.*, 2000). Crystallographic studies involving four different mannopyranoside-mimicking peptides suggested that the predominant interactions involving these peptides (and shared by the mannopyranoside moiety) in their binding to the common receptor concanavalin A (ConA) were van der Waals forces and hydrophobic features (Jain *et al.*, 2001*a*). On the other hand, the crystal structure of ConA bound to *meso*-tetrasulfonatophenylporphyrin (H_2TPPS) suggested that the predominant contribution to molecular mimicry involving the porphyrin molecule and mannopyranoside on binding ConA was through hydrogen bonding (Goel *et al.*, 2001). Thus, three chemically independent ligands provided interesting facets of molecular mimicry on binding to ConA.

Porphyrin derivatives are cofactors of many proteins. The structure of porphyrin is remarkably pluripotent in terms of chemical features that can facilitate interactions with proteins and hence is a favorable candidate for molecular-recognition studies. It has been shown that H_2TPPS, a porphyrin derivative binds to a variety of different lectins *e.g.* jacalin, snakegourd lectin, ConA and *Trichosanthes cucumerina* lectin (Bhanu *et al.*, 1997; Komath, Bhanu *et al.*, 2000; Komath, Kenoth *et al.*, 2000; Kenoth *et al.*, 2001). H_2TPPS binds to jacalin with an affinity ($K_a = 0.65 \times 10^4\ M^{-1}$) comparable to that of galactopyranoside, its specific carbohydrate ligand. Binding of the porphyrin to jacalin has also been observed in the presence as well as in the absence of galactopyranoside. Jacalin is a plant lectin with known specificity for T-antigen, galactose, mannopyranoside and a variety of other sugars (Jeyaprakash *et al.*, 2002; Sankarnarayan *et al.*, 1996; Bourne *et al.*, 2002; Wu *et al.*, 2003). Based on the crystallographic and thermodynamic studies of many jacalin–carbohydrate complexes, it has been established that the ligand-binding site of jacalin exhibits plasticity (Bourne *et al.*, 2002; Swaminathan *et al.*, 2000). On the other hand, it has been shown that jacalin has the ability to discriminate between different carbohydrate analogues in terms of binding affinities on the basis of subtle differences between them (Jeyaprakash *et al.*, 2002). It was therefore considered that the interactions of jacalin with diverse ligands would provide an interesting model towards further understanding of molecular mimicry at the structural level. It was pertinent to address whether the mimicry of mannopyranoside by H_2TPPS arises from structural correlation between the specific sugar and the porphyrin moiety and whether it would be manifested irrespective of the receptor with which they interact.

In the present study, we have determined the structure of H_2TPPS in complex with jacalin at 1.8 Å resolution. The structure shows that although the porphyrin binds at the carbohydrate-binding site, it does not occupy the cavity in which sugar binds. Instead, it covers this cavity, with the sulfonatophenyl side groups of the porphyrin fitting snugly into the grooves on the surface of jacalin.

Table 1
Data-collection, structure solution and refinement statistics.

Values in parentheses are for the last resolution shell (1.86–1.80 Å).

Unit-cell parameters (Å)	$a = 46.1$, $b = 101.9$, $c = 107.7$
Space group	*I*222
Maximum resolution (Å)	1.8
Completeness (%)	87.9 (79.9)
No. of observed reflections	115569
No. of unique reflections	23948
Multiplicity	4.8
Average $I/\sigma(I)$	10.8 (1.0)
R_{merge} (%)	6.1 (61.3)
No. of solvent atoms	57
Solvent content (%)	67.9
R.m.s.d. bond lengths (Å)	0.01
R.m.s.d. bond angles (°)	1.4
R_{cryst} (%)	21.9 (42.6)
R_{free} (%)	23.3 (43.8)

2. Materials and methods

2.1. Preparation of jacalin–H_2TPPS complex crystals

Jacalin was purified by affinity chromatography on a guar-gum column and eluted with galactose according to a previously detailed protocol (Komath, Bhanu *et al.*, 2000). The purity of the eluted protein was assessed by plain polyacrylamide-gel electrophoresis, where it yielded a single band. 10 mg ml^{-1} jacalin and 0.3 mg ml^{-1} H_2TPPS (Alfa Inorganics, USA) were solubilized in 50 m*M* phosphate buffer pH 7.2. The porphyrin and jacalin were co-crystallized using the hanging-drop method. 5 µl of reservoir solution was added to a 5 µl drop containing the jacalin–porphyrin mixture in a molar ratio of 2:1. The reservoir solution contained 0.5 *M* $(NH_4)_2SO_4$, 0.25 *M* NaCl in 50 m*M* phosphate buffer pH 7.2. Crystals appeared after about three weeks.

2.2. X-ray diffraction data collection

The X-ray intensity data were collected on an image-plate detector (MAR Research, Germany) installed on a rotating-anode X-ray source (Rigaku, Japan) which was operated at 40 kV and 70 mA (Cu $K\alpha$ radiation). The crystal-to-detector distance was 110 mm and 1° oscillation frames were recorded at room temperature. The diffraction data were collected from a single crystal that diffracted to beyond 1.8 Å resolution. The data were processed using *DENZO* (Otwinowski, 1993) and subsequently scaled using *SCALEPACK*. The data-collection statistics are shown in Table 1.

2.3. Structure solution and refinement

The jacalin–H_2TPPS complex crystallizes in space group *I*222, which has not been reported previously for jacalin. Molecular-replacement calculations were carried out using the program *AMoRe* (Navaza, 1994). The monomer of jacalin

(PDB code 1jac) was used as a model for rotation/translation-function calculation in the resolution range 8–4 Å, which gave a correlation coefficient of 65% and an R factor of 31.4%. Examination of the crystal packing showed no evidence of steric clashes. The model was subjected to refinement using *CNS* (Brünger *et al.*, 1998). The Matthews coefficient (V_M) was calculated to be 3.8 Å^3 Da^{-1}. The solvent content was estimated to be 67.9%. The $F_o - F_c$ map after rigid-body refinement showed excellent electron density for the porphyrin ligand. Conjugate-gradient minimization and restrained individual B-factor refinement were carried out. Water molecules were picked up using an $F_o - F_c$ map with an electron-density cutoff of 2.5σ. After several rounds of refinement, R_{cryst} and R_{free} converged to 21.9 and 23.3%, respectively, in the resolution range 100–1.8 Å. The statistics of the final refined model are shown in Table 1.

2.4. Buried surface area and interaction-energy calculations

The buried surface area and the interaction-energy calculations were performed using the HOMOLOGY and DOCKING modules of the *MSI* software (Molecular Simulations Inc.).

3. Results and discussion

3.1. Overall structure and interactions in the jacalin–porphyrin complex

The jacalin–H_2TPPS complex crystallizes in space group *I*222. Interestingly, none of the jacalin-containing crystal structures determined so far have exhibited this symmetry (Jeyaprakash *et al.*, 2002; Sankarnarayan *et al.*, 1996; Bourne *et al.*, 2002). The molecular packing of jacalin complexed with porphyrin in the unit cell, as viewed along the crystallographic a axis, is shown in Fig. 1. The jacalin molecule exists as a tetramer of about 66 kDa. Each jacalin monomer is made up of two chains, chain A consisting of 133 residues and a smaller chain B consisting of 20 residues (Mahanta *et al.*, 1992). All the residues of chain A were clearly defined, while only residues 3–18 of chain B could be traced in the electron-density map. The binding of the porphyrin molecule does not seem to cause any major changes in the backbone conformation of jacalin. A stacked pair of porphyrin molecules together with a monomer of jacalin form the asymmetric unit. This porphyrin pair is sandwiched between two symmetry-related jacalin monomers. The interaction of the porphyrin pair with two monomers of jacalin from two independent tetramers leads to an extensive cross-linking of the lectin in the crystals of the complex. The porphyrin has previously been shown to bring about cross-linking of ConA molecules in the crystal structure of the ConA–H_2TPPS complex (Goel *et al.*, 2001). Similar cross-linking has been previously observed in the case of the complex of T-antigen with jacalin, in which the Gal moiety of the T-antigen disaccharide interacts with the residues of the adjacent tetramers (Jeyaprakash *et al.*, 2002).

H_2TPPS is a free-base porphyrin, having four aryl side groups attached to a closed tetrapyrrole ring known as the porphine core. Each aryl side group is made up of a phenyl ring with a sulfonate group attached at the *para* position. In the asymmetric unit of the jacalin–porphyrin complex crystal structure, H_2TPPS exists in its dimeric state, with the stacked pair of two porphyrin molecules being staggered and translated with respect to each other. The phenyl rings of the two molecules are not coplanar with the porphine ring; instead, they are rotated by varying degrees with respect to the porphine core. The angle of this rotation ranges from 65 to 105° among the various phenyl rings. Stacking of porphyrin molecules has been observed previously in other protein–porphyrin complexes, which include bacteriochlorophyll, a molecule in the light-harvesting complex from *Rhodopseudomonas acidophila* (McDermott *et al.*, 1995), bacteriochlorophylls forming a primary electron donor 'special pair' (Michel *et al.*, 1986) and the ConA–H_2TPPS complex (Goel *et al.*, 2001). The stack of two porphyrins in the asymmetric unit of the crystal interacts with the known carbohydrate-binding site of the jacalin monomer. One of these porphyrin molecules (porphyrin I), binds to jacalin such that it covers the carbohydrate-binding cavity of the jacalin molecule. The $2F_o - F_c$ electron density of the porphyrin I contoured at 1.0σ is shown in Fig. 2. The other porphyrin molecule of the pair (porphyrin II), by virtue of packing between the symmetry-related molecules, interacts with the symmetry-related jacalin molecule at a site not equivalent to the porphyrin I binding site. The two sites are about 20 Å away from each other on the protein surface.

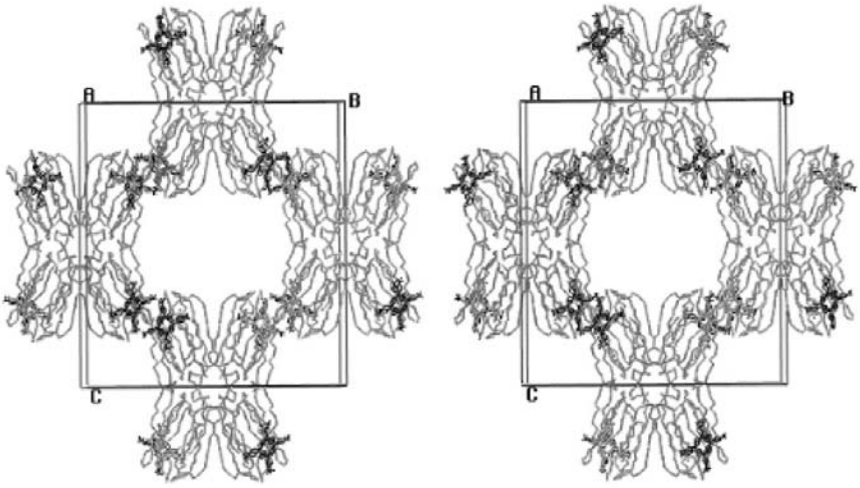

Figure 1
Stereo drawing of the molecular packing of jacalin–H_2TPPS in the unit cell as viewed along the crystallographic a axis. A stack of porphyrin molecules interacting with two independent molecules of jacalin leads to extensive cross-linking of the jacalin molecules.

Porphyrin I, occupying the carbohydrate-binding site on jacalin, interacts with nine residues of the lectin (Fig. 3). Seven water molecules are also found within the van der

Waals distance of this porphyrin molecule. N atom N8 of the porphine ring forms a hydrogen bond with a water molecule, W16, in addition to forming a hydrogen bond with Gly1 N. The N atom of the opposite pyrrole ring (N4) hydrogen bonds to the Gly1 O atom of the jacalin molecule. O atom O9 of the sulfonate group of this porphyrin forms a hydrogen bond with one of the seven water molecules (W23), which in turn forms a hydrogen bond with Ser76 OG. Another O atom, O10, directly hydrogen bonds to Ser76 O of jacalin. Porphyrin II shows van der Waals interactions with seven amino acids of the symmetry-related jacalin molecule, in addition to the stacking interactions with porphyrin I. Tyr78 is the only residue of the jacalin molecule that lies within van der Waals distance of porphyrin II; all other interactions are with the symmetry-related jacalin molecule. Interaction of porphyrin II with jacalin involves residues in the loop 50–53 and another loop consisting of three residues 19–21. Only two bound water molecules (W36 and W29) are found in the vicinity of porphyrin II. W29 mediates hydrogen bonds between residue Tyr19 OH of the protein and the O11 and O13 atoms of the sulfonate group of the H_2TPPS molecule.

Thus, the two porphyrin molecules in the stacked pair show different interactions with jacalin. The interactions of porphyrin I lead to the burial of a larger surface area of the protein molecule compared with those of porphyrin II. About 341.8 Å^2 of the surface area of jacalin becomes solvent-inaccessible owing to porphyrin I occupying the carbohydrate-binding site. The porphyrin II interaction leads to 226.7 Å^2 of surface area of the symmetry-related jacalin becoming solvent-inaccessible. We have also compared the interaction energy of each porphyrin molecule with jacalin independently, in which porphyrin I interacts with the carbohydrate-binding site whereas porphyrin II is associated with the symmetry-related molecule. The total energy of interaction for porphyrin I was −240 kJ mol^{-1} and that for porphyrin II was −159 kJ mol^{-1}. These energies are consistent with the observed buried surface areas in the two cases. Although both the porphyrins show van der Waals contacts with a similar number of protein residues, the porphyrin I interaction involves significantly more water molecules. Also, the sulfonate O atom in both porphyrins forms direct or water-mediated hydrogen bonds to the protein atoms. In addition to these hydrogen bonds, the pyrrole-ring N atoms of porphyrin I form additional direct and water-mediated hydrogen bonds to the protein residues near the carbohydrate-binding site. Thus, binding of the pair of porphyrins is brought about by the interactions of porphyrin I at the carbohydrate-binding site of one jacalin molecule and the additional interactions of porphyrin II with the symmetry-related jacalin monomer, resulting in the cross-linking of jacalin molecules and perhaps facilitating crystal stability.

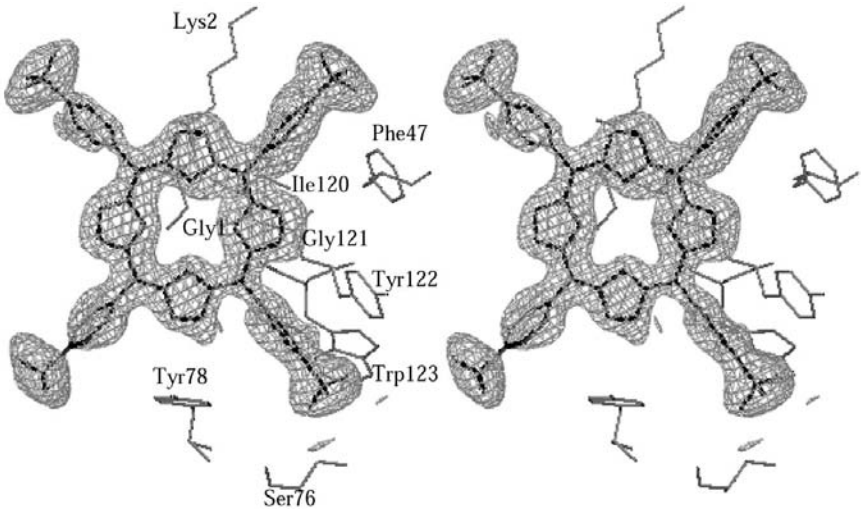

Figure 2
Stereo diagram of the $2F_o - F_c$ electron density of porphyrin I at the ligand-binding site of jacalin contoured at 1.0σ. The density shows clear evidence of rotation of the phenyl rings with respect to the porphine core. The porphyrin molecule (black) has been built into the map. The interacting residues of the jacalin molecule are labelled.

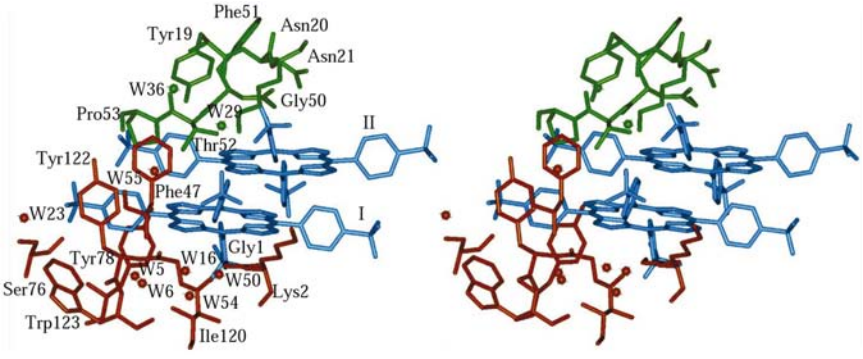

Figure 3
Stereo drawing of the stacking of porphyrin molecules (blue) with the interacting amino acids in jacalin. The protein molecule interacting with porphyrin I is depicted in brown, whereas the residues of the symmetry-related molecule of jacalin interacting with porphyrin II are shown in green. The amino acids are marked using three-letter codes and the water molecules are labeled W.

3.2. Comparison of porphyrin binding with the carbohydrate ligands

The structure of the jacalin–H_2TPPS complex was compared with that of native jacalin and its complexes containing galactopyranoside, mannopyranoside and T-antigen (Jeyaprakash *et al.*, 2002; Sankaranarayan *et al.*, 1996; Bourne *et al.*, 2002). Although large backbone conformational changes are not seen in jacalin on porphyrin binding, certain side chains exhibit conformational reorientations. The side chain of Phe47 shows a distinct change (a conformational flip) when bound to H_2TPPS. This conformation of this side chain is not observed in any other complexes of jacalin.

The reorientation of the Phe47 side chain opens up the binding cavity on jacalin and makes room for the porphyrin molecule to be accommodated. The side-chain orientation of Tyr78 in the H_2TPPS–jacalin complex structure, although very similar to that in complex with the carbohydrate ligands of jacalin, is different from that observed in the native protein structure. The orientation of yet another side chain, Tyr122, in the porphyrin–jacalin complex is similar to that in the complexes with mannose and galactose, but is different from that of the native structure or in complex with T-antigen. Thus, the three aromatic residues Phe47, Tyr78 and Tyr122 reorient themselves away from the binding cavity, creating space for porphyrin to bind at the carbohydrate-binding site (Fig. 4*a*).

Table 2
Hydrogen-bonding equivalence of the porphyrin–jacalin complex to the galactopyranoside–jacalin complex and native jacalin.

Galactose (1jac) (distance in Å)	Jacalin	H_2TPPS (distance in Å)	Native (1ku8) (distance in Å)
O5 (2.95)	Tyr122 N	—	W389 (3.35)
O6 (2.92)	Trp123 N	W6 (3.07)	W390 (2.86)
O6 (3.03)	Trp123 O	W6 (3.20)	W390 (3.11)
O6 (3.24)	Tyr122 N	W6 (2.92)	W390 (3.17)
O6 (2.83)	Asp125 OD1	W6 (2.74)	W390 (2.95)
O4 (3.22)	Gly1 N	W16 (3.32)	W387 (3.01)
O4 (2.84)	Asp125 OD1	W16 (2.80)	W387 (2.81)
O4 (3.00)	Asp125 OD2	W16 (3.13)	W387 (3.27)
O3 (2.76)	Gly1 N	N8B (3.36)	—

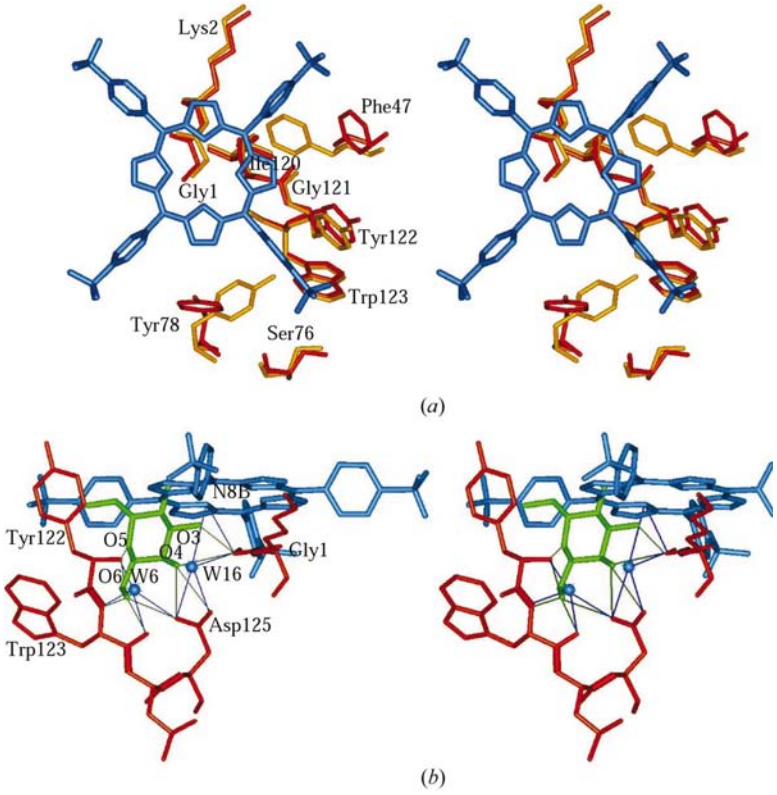

Figure 4
Binding of H_2TPPS at the carbohydrate-binding site of jacalin. (*a*) Stereo drawing of the conformational changes seen in the jacalin (brown) molecule on porphyrin (blue) binding. The corresponding side-chain conformations in the absence of porphyin seen in case of native jacalin are depicted in yellow. (*b*) Stereo drawing of the hydrogen-bonding correspondence between the porphyrin and the galactopyranoside ligands of the jacalin protein. The respective hydrogen bonds are shown in ligand colours.

While two residues, Tyr78 and Tyr122, show stacking interactions with the phenyl rings of the porphyrin molecule, only Tyr78 has been shown to exhibit stacking interactions with the pyranoside ring in the case of the carbohydrate ligands (Jeyaprakash *et al.*, 2002). Four aromatic residues, Phe47, Tyr78, Tyr122 and Trp123, have been implicated in defining the galactose-specificity of the jacalin molecule (Pratap *et al.*, 2002). Interestingly, all of these except Trp123 are involved in redefining the porphyrin-binding site. In addition to the stacking interactions, H_2TPPS also forms hydrogen bonds, some of which involve water bridges, with jacalin at the carbohydrate-binding site.

The N atom of the pyrrole ring (N8B) is hydrogen bonded to Gly1 N. It also forms another relatively weak (3.36 Å) hydrogen bond with a water molecule, W16. W16 and W6 correspond to two of the three water molecules present in the carbohydrate-binding site of native jacalin. The three water molecules effectively mimic the interactions of the carbohydrates in the ligand-free state and are displaced on binding carbohydrate ligands. They mimic the entire hydrogen-bonding network of the galactose except for one hydrogen bond between O3 of the sugar and Gly1 N, replicating eight out of the nine hydrogen bonds made by galactose. However, two of these water molecules (W16 and W6) are retained while the third is absent in the porphyrin–jacalin complex. These two water molecules in the porphyrin–jacalin complex still mediate seven hydrogen bonds with the protein molecule while also interacting with the porphyrin molecule. Thus, there appears to be only one additional hydrogen bond in the galactopyranoside-bound state. The correspondence of the hydrogen-bonding network at the carbohydrate binding site of jacalin, as seen in the native, galactopyranoside-bound and porphyrin-bound jacalin structures, is shown in Table 2. The geometrical relationship of the hydrogen-bonding network of the porphyrin–jacalin and galactopyranoside–jacalin complexes is compared in Fig. 4(*b*). The interactions of porphyrin with jacalin are relatively weak compared with those observed between galactopyranoside and jacalin, perhaps because the former involves largely water-mediated hydrogen bonds. The nature of the atoms involved in these hydrogen bonds may also be responsible for their weakness. In general, N—H···N hydrogen bonds are expected to be energetically weaker than O—H···N hydrogen bonds. Thus, the porphyrin binding of jacalin exemplifies the adaptability of the carbohydrate-binding site of jacalin as mediated by flexible side-chain orientations and cementing by water molecules.

Jacalin is known to bind to T-antigen with a significantly higher affinity than to galactopyranoside. The binding of T-antigen (Galβ1-3GalNAc) to jacalin differs from that of galactopyranoside mainly

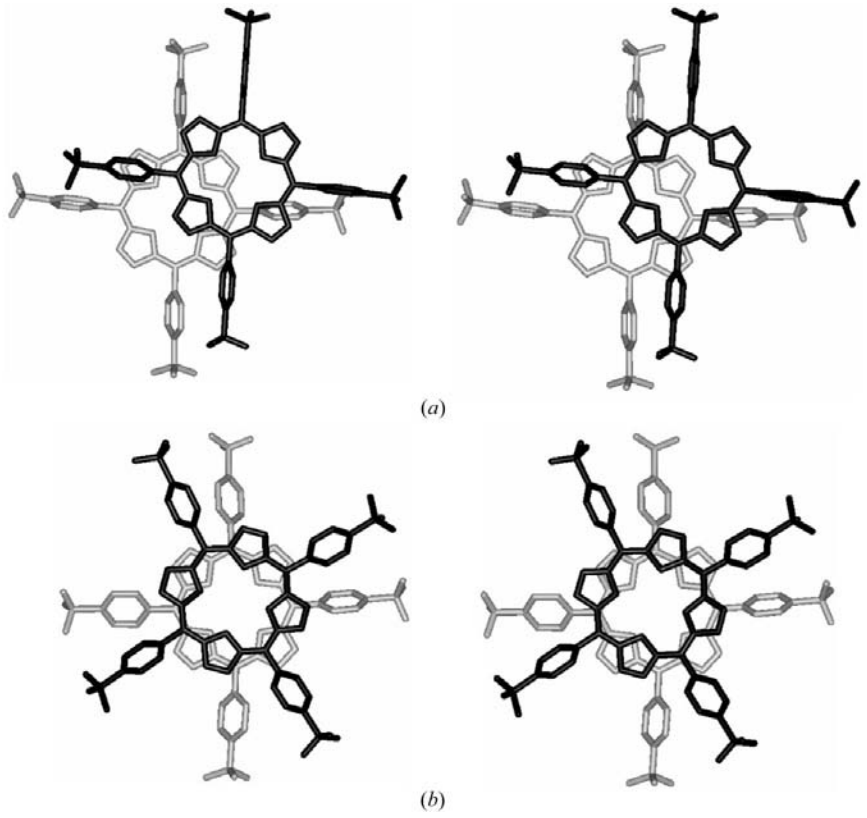

Figure 5
Stereo drawing of the porphyrin dimer stack as seen in (*a*) the jacalin–H_2TPPS complex and (*b*) the ConA–H_2TPPS complex. The two porphyrins are represented as black and grey sticks.

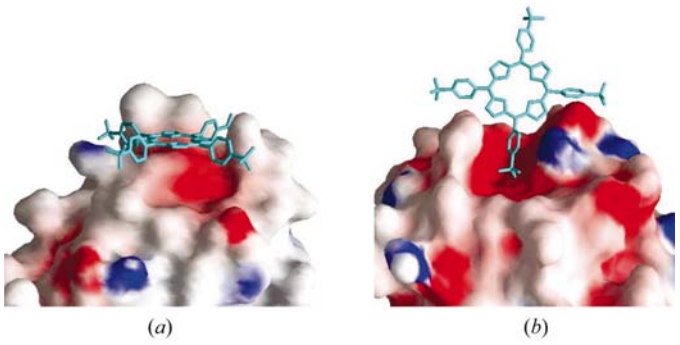

Figure 6
Comparison of porphyrin juxtaposed with (*a*) jacalin and (*b*) ConA in the corresponding carbohydrate-binding sites. Porphyrin is shown in sticks (blue) and jacalin and ConA, incorporating the corresponding sandwiched water molecules, are molecular-surface representations coloured according to charge (red, negative; blue, positive).

in the additional interactions of the Gal moiety mediated by two bound water molecules (Jeyaprakash *et al.*, 2002). These water molecules are also seen bound to the protein molecule in the porphyrin–jacalin complex, but do not show any interaction with the porphyrin molecule. Thus, the mimicry of the hydrogen-bonding pattern of the carbohydrate ligands of jacalin by the porphyrin molecule is limited to the monosaccharide-binding site on jacalin and does not involve the disaccharide-binding site.

3.3. Comparison of H_2TPPS binding to two different receptors: jacalin and ConA

The binding of H_2TPPS at the carbohydrate-binding site of ConA as well as that of jacalin resulted in extensive cross-linking of the lectins in their respective crystal structures. The cross-linking in the ConA–H_2TPPS complex was effectively brought about by the symmetric interactions of two stacked porphyrins with the carbohydrate-binding site of four independent monomers of ConA. On the other hand, in the jacalin–H_2TPPS complex the cross-linking is facilitated by the interactions of the stacked pair of H_2TPPS with the symmetry-related jacalin monomer. A comparison of the modes of porphyrin stacking in the two complexes is shown in Fig. 5. In the case of ConA, the porphine cores of the two porphyrins are stacked such that they are rotated with respect to each other but not translated.

The ConA molecule does not undergo any major conformational changes on binding the porphyrin molecule. On the other hand, jacalin shows substantial side-chain reorientations in accommodating the porphyrin molecule, thus exhibiting considerable plasticity in the binding site. The porphine core of the porphyrin directly interacts with jacalin, but this is not the case with ConA. While H_2TPPS interacts with ConA primarily through hydrogen bonds that mimic those of the carbohydrate ligand, it interacts with jacalin showing dominant contribution of stacking interactions involving the aromatic residues Tyr78 and Tyr122 in the carbohydrate-binding cavity of the lectin in addition to the hydrogen bonds. Although H_2TPPS binds to both ConA and jacalin by interacting at their carbohydrate-binding sites, it does not enter the carbohydrate-binding cavity of jacalin but rather perches on top of the binding site. Three of the four sulfonatophenyl side groups of the porphyrin molecule fit over the crevices of the jacalin surface. On the other hand, only a single sulfonatophenyl group of H_2TPPS interacts with a monomer of ConA. Two of the side groups of this porphyrin molecule interact with two independent but crystallographically related monomers of ConA, while the two other side groups interact with the solvent. The mode of porphyrin binding to jacalin and ConA is depicted in Figs. 6(*a*) and 6(*b*), respectively.

In its interactions with ConA, the H_2TPPS molecule mimics the hydrogen-bonding pattern of the mannopyranoside with the help of bound water molecules. Two O atoms of the sulfonatophenyl group of the porphyrin molecule mimic two of the hydrogen bonds observed in the mannopyranoside–ConA complex; one of these is a direct hydrogen bond to the protein, while the other is a water-mediated hydrogen bond. In

contrast, the H_2TPPS molecule shows only one direct hydrogen bond through the pyrrole-ring N atom N8B to Gly1 N, which corresponds to the galactopyranoside interaction with jacalin. Thus, the mimicry of the hydrogen bonds of the galactopyranoside–jacalin complex by H_2TPPS seems to be partial when compared with the mimicry of hydrogen bonds as seen in the porphyrin–ConA complex. This is partly owing to the nature of the atoms of the porphyrin involved in the hydrogen-bonding network. The N—H···N bonds involved in mimicking the carbohydrate ligand in the H_2TPPS–jacalin complex are weaker than the hydrogen bonds involving the O atom of porphyrin formed in the complex with ConA. Another factor that might contribute is the observation that the porphyrin molecule in complex with jacalin does not enter the carbohydrate-binding cavity, unlike in its complex with ConA. Thus, the mode of interaction of H_2TPPS is substantially different when binding to these two lectins, although it occupies the carbohydrate-binding site in both cases. While the porphyrin binding is mediated by exactly replicating the hydrogen-bonding network of mannopyranoside through the sulfonate atoms in case of ConA, it is the plasticity associated with carbohydrate-binding site that accommodates the porphyrin molecule in the case of jacalin.

4. Conclusions

The physico-chemical properties and the size of the H_2TPPS molecule are such that it can provide a diverse set of possible independent interactions on binding to a protein receptor. The O atoms of the sulfonate groups can participate in hydrogen-bonding interactions and mimic the properties of the hydroxyl atoms. The four phenyl rings and the central tetrapyrrole porphine ring generate the hydrophobic interaction component of the porphyrin system. The free rotation available to the phenyl rings facilitates the formation of stacking interactions with the aromatic residues of the protein. The porphine core of the porphyrins can stack together in different ways and this, coupled with the symmetric and the multivalent nature of the porphyrin molecule, can lead to multifarious interaction potencies, forming stabilized protein–porphyrin complexes. This is consistent with the fact that the accumulation of porphyrins in tumours is thought to be governed by their affinity for proteins, which results from their enriched π electrons and amphipathic properties owing to this stacking phenomenon (Nakajima *et al.*, 1999).

The binding of porphyrin at the carbohydrate-binding site in the present crystal structure apparently contradicts the suggestions of simultaneous binding of carbohydrate and porphyrin to jacalin based on earlier biochemical studies (Komath, Bhanu *et al.*, 2000). However, the natural ligands of lectins are complex carbohydrates containing repetitive monomeric units and are not simple monosaccharides. Thus, the binding sites for the recognition of such natural carbohydrates may be much larger, comprising several subsites for monosaccharides (Jain, Kaur, Sundaravadivel *et al.*, 2000). Therefore, even if the primary monosaccharide-binding site is saturated with porphyrin in solution, sugar moieties can still bind to other secondary subsites on the lectin.

The interaction of porphyrin with jacalin through water bridges and the adjustments of side-chain conformations to accommodate porphyrin in the carbohydrate-binding site represents another facet of molecular mimicry. The water molecules in the binding site of jacalin act like cement in that they are displaced on recognizing some ligands, while they mediate interactions with others. The combination of the conformational changes brought about in the jacalin molecule and the hydrogen-bonding interactions of the porphyrin suggest that the porphyrin binding resembles the binding of galactopyranoside and mannopyranoside more than the binding of T-antigen. The porphyrin molecule binds to two different lectins using overlapping but not identical sets of interaction forces. The structural basis of peptide–mannopyranoside mimicry has been defined in terms of the plasticity of interactions dominated by shape and hydropathy features and has been explained in terms of the equivalence of hydrogen bonding alone in the case of mannopyranoside–porphyrin mimicry in ConA. In the present case in which galactopyranoside and porphyrin are being compared, a combination of the two facilitates the mimicry.

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