acid/base glutamate hydrogen-bonds to the glycosidic oxygen; and the conserved asparagine hydrogen-bonds to the C2-hydroxyl near the cleavage site. A close approach of two key glutamate residues provides an elegant mechanism for the shift in the pK_a of the acid/base for the glycosylation and deglycosylation half-reactions. The structure also identifies and defines the roles of five further residues which are well-conserved within the superfamily. The structure is entirely consistent with a large body of kinetic data observed for wild-type and mutated forms of superfamily members, and allows us to extend the known chemical mechanism with a detailed sequence of physical steps that we propose are involved in catalysis by the enzymes. This superfamily includes a large number of cellulases, so the insights will aid protein engineering efforts to improve cellulase activities for use in biomass conversion.

PS04.11.10 PRELIMINARY X-RAY STUDY OF TETRACARPIDIUM CONOPHORUM AGGLUTININ II, AN ISOLECTIN FROM THE NIGERIAN WALNUT. Tracey Barrett, Kim Henrick, Guy Dodson, Theresa Animashaun, Colin Hughes, National Institute For Medical Research, Mill Hill, London, UK

Lectins form a group of structurally diverse proteins that bind to specific oligosaccharide sequences. They occur in almost all living organisms and despite having their roles well characterised in mammals are of largely unknown function in plants.

Two isolectins (TCAI and TCAII) have been isolated from seed extracts of the Nigerian walnut (*Tetracarpidium conophorum*). Both TCAI and TCAII are glycosylated and have the respective molecular weights of 70 and 30kda. TCAI exists as a disulphide linked dimer and TCAII as a monomer. Both isolectins have specificity for oligosaccharides with terminal galactose residues consistent with this lectin family which includes Ricin and Ricinus communis agglutinin.

Orthorhombic crystals of TCAII were obtained (space group $P2_12_12_1$ with cel dimensions a=65.7Å, b=86.3Å, c=1 18.3Å and two molecules in the assymetric unit) which diffracted beyond 2.4Å. It was possible to locate the position of both molecules in the unit cell using molecular replacement with a search model consisting of the Ricin B-chain. The overall fold of TCAII is very similar to that of Ricin where the molecule can be divided into two globular domains which are formed from a series of disulphide linked gamma loops (there is little significant secondary structure). TCAII was crystallised in the presence of lactose and it is possible to identify electron density for at least galactose in both sugar binding sites. The structure is currently undergoing refinement.

References:Animashaun, T., Togun, R.A. and Hughes, R.C., (1994) *Glycoconjugate Journal*, 11, 299-303.

PS04.11.11 SUGAR IN YOUR GARDEN/DIET: LECTIN/SUGAR COMPLEXES. P.J. Rizkallah (Daresbury), C.D. Reynolds (John Moores University, Liverpool), S.D. Wood (JMU), L.M. Wright (JMU), R. Kelly (JMU), Pei-Wen Lei (JMU), R. Loris (Free University, Brussels), A.K. Allen (Imperial College)

1. Flower Bulb Lectins

The mannose binding lectins from flower bulbs were found to have antiviral activity, most importantly against HIV, by recognising the glycoprotein GP120 on its surface. This property has been exploited in the purification of HIV virions. The structures of these lectins from amaryllis and bluebell bulbs were solved, in order to characterise their interaction with mannose, and also as a first step towards solving the structure of the complex with GP120. Molecular Replacement (MR) experiments showed that as little as 4% of the total scattering material was sufficient to solve the amaryllis lectin structure, using a model of the snowdrop lectin. A difference electron density map revealed a mannose bound to each of the two molecules in the asymmetric unit. The overall packing showed tetrameric clusters with pseudo 222 symmetry, where 2 asymmetric units provided a dimer each. Refinement was carried out at 2.3 Å. The bluebell lectin is highly similar, in affinity and other properties, to the amaryllis lectin. It was crystallised in an orthrhombic space group, with and without mannose, and both forms diffracted to 1.85 Å. Using the refined model of the amaryllis lectin, the structure was solved with MR. The packing was slightly different, possibly due to the extra protein sequence in this lectin. Refinement is currently underway.

2. Legume Lectins

Unbound and complexed Lentil lectin has been crystallised and the structure refined at high resolution in three different crystal forms (all at 1.5Å). Seeds of leguminous plants use lectins to store specific sugars. Although lectin structures are similar, their specificity varies according to species. The gene structure is common among all, with a high degree of homology. The binding site motif is also common, with side chain mutations determining the specificity. Binding is indirectly mediated by two metal binding sites close to the saccharide site, stabilising an unusual cispeptide bond, important for sugar recognition. The unbound lectin crystallised in two different space groups which diffracted to a resolution higher than 1.4 Å. Data were collected up to 1.5 Å. The sucrose complex crystallised in a third space group, and also diffracted to 1.5 Å resoltuion, when cryo-cooled. The differences between the three structures were mainly due to the packing arrangement.

PS04.11.12 A MUTANT SHIGA-LIKE TOXIN IIv BOUND TO ITS RECEPTOR. Hong Ling*, Amechand Boodhoo$, Glen D. Armstrong$, Clifford G. Clark#, James L.Brunton# and Randy J. Read*$ *Department of Biochemistry & $Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, T6G 2H7, Canada, #Department of Microbiology and Medicine, University of Toronto, Toronto Ontario M5S 1A1, Canada

Shiga-like toxin II variant (SLT-IIv) is a member of the Shiga toxin family. SLT-IIv is produced by certain strains of *E.coli* that cause edema in pigs. Like other family members, it is a bipartite molecule composed of an enzymatic (A) subunit, and five copies of a binding (B) subunit. The B pentamers of Shiga-like toxins mediate receptor binding, cytotoxic specificity and extracellular localization of the holotoxin. The functional receptor of the B subunits for most family members is the glycolipid Gb_3 (globotriaosyl ceramide), but SLT-IIv has a preference for the glycolipid Gb_4 (globotetraosyl ceramide). Interestingly, a double mutant of SLT-IIv (designated as GT3: Gln65/Glu, Lys67/Gln in the B subunit) loses its preference for Gb_4 and instead binds most strongly to Gb_3.

In order to understand the molecular basis for the receptor specificity, we have determined the structure of the GT3 mutant B pentamer complexed with Gb_3 at 2Å. The structure was solved by molecular replacement using the Shiga-like toxin I B subunit as a search model (64% identity with the SLT-IIv B subunit). Refinement consisted of XPLOR runs combined with 5-fold averaging in DEMON, and manual rebuilding in O. The refined structure has excellent stereochemistry and an R-factor of 17.5% (R_{free}=22.8%).

The B subunit structure has a typical oligomer binding (OB) motif which consists of a five-stranded antiparallel β-barrel capped by an alpha helix. The five identical B subunits form a symmetric pentamer. The structure reveals two Gb_3 binding sites per monomer on the bottom surface (opposite to the interface with the A subunit) of the B pentamer.

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