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 pKα of the acid/base for the glycosylation and deglycosylation half-reactions. 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 orthorhombic 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 1.5 Å). 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 resolution, when cryo-cooled. The differences between the three structures were mainly due to the packing arrangement.

PS04.11.12 A MUTANT SHIGA-LIKE TOXIN IIB BOUND TO ITS RECEPTOR, Hong Ling*, Amechand Boodhoo*, Glen D. Armstrong5, Clifford G. Clark5, James L. Brunnou5 and Randy J. Read5 5Department of Biochemistry & Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, T6G 2H7, Canada, 1Department 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 Gb3 (globotriaosyl ceramide), but SLT-IIv has a preference for the glycolipid Gb4 (globotetraosyl ceramide). Interestingly, a double mutant of SLT-IIv (designated as GT3: Gb6/Glu, Lys67/Gln in the B subunit) loses its preference for Gb4 and instead binds most strongly to Gb3.

In order to understand the molecular basis for the receptor specificity, we have determined the structure of the GT3 mutant B pentamer complexed with Gb4 at 2 Å. The structure was solved by molecular replacement using the Shiga-like toxin I B subunit as a search model (64% identity with the SLT-IV 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% (Re=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 Gb4 binding sites per monomer on the bottom surface (opposite to the interface with the A subunit) of the B pentamer.

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