substrate analogue is particularly interesting since it provides the first glimpse of an intact oligosaccharide bound across the active site of an enzyme. The pyranaose ring is distorted into a boat conformation which gives a pseudo-axial conformation for the glycosidic bond and leaving group as predicted by David Phillips in 1974 and as expected on stereoelectronic grounds.

**MS04.11.06 THE STRUCTURE OF THE AMINO-TERMINAL IG-LIKE SIALIC ACID BINDING DOMAIN OF SIALOADHESIN.** A.May[1], R.C.Robinson[1], P.Bradfield[2], M.Vinson[2], P.R.Crocker[2], E.Y.Jones[1]. [1]Laboratory of Molecular Biophysics, University of Oxford, Oxford. U.K; [2]I.C.R.F. Laboratories, University of Oxford, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford. U.K.

A functional fragment of Sialoadhesin, which recognises oligosaccharides terminating in NeuAcα2-3Gal in N- and O-linked glycans, has been crystallised, and its structure has been solved to 2.6Å resolution using MAD. Sialoadhesin is a macrophage-restricted receptor containing 17 Immunoglobulin(Ig)-like domains, of which the N-terminal domain is necessary and sufficient to mediate sialic-acid dependent binding (1). The structure consists of a single V-set Ig domain, containing 115 amino acids. The characteristic Ig inter sheet disulphide bridge is replaced by an intra-sheet disulphide between the B and E strands. The structure most closely resembles a monomer of CD8α, with loops surrounding the binding site identified by site-directed mutagenesis (2). Residues implicated in sialic acid binding are found on the G-F-C-C'-C" face. This face also forms the interactive surface in CD2 and VCAM-1, other cell surface members of the IgSF. For MAD phasing, data sets were collected at three wavelengths from a single crystal flash-frozen at 104K. The crystals belong to space group $P3_121$ with unit cell dimensions of a = b = 38.9, c = 152.6, α = β = 90°, γ = 120°, with one molecule in the crystallographic asymmetric unit.

(1) Nath., D., van der Merwe, P.A., Kelm, S., Bradfield, P. and Crocker, P.R. (1995) *J.Biol.Chem.* vol.270 no.44 pp.26184-26191
(2) Vinson, M., van der Merwe, P.A., Kelm, S., May, A., Jones, E.Y., and Crocker, P.R., (1996) *J.Biol.Chem.* (in press)

**MS04.11.07 RHAMNOGALACTURONASE A FROM *ASPERGILLUS ACULEATUS*. A RIGHT-HANDED β-HELIX FOLD.** By Thomas Nordahl Petersen and Sine Larsen, Centre for Crystallographic Studies, University of Copenhagen, Denmark

The crystal structure of this plant cell wall degrading enzyme has been determined to 2.0 Å resolution, using the SIRAS method.

Pectin is the major component of a plant cell wall. It consist of a smooth region of homogalacturonan and a 'hairy region' of rhamnogalacturonan. The backbone of rhamnogalacturonan is composed of a chain of alternating rhamnose and galacturonic acid residues α-L-Rha(1-4)-α-D-GalUA( 1-2). Rhamnogalacturonase A hydrolyses the glycoside bonds α-D-GalUA(1-2)-α-L-Rha. The minimum size of the substrate has been found to be a 12'mer, resulting in a 5'mer and a 7'mer after enzymatic hydrolysis. Recombinant enzyme has been obtained from an overexpression system set up in *Aspergillus oryzae*. The enzyme crystallizes in space group I222 with one molecule in the asymmetric unit. The three dimensional fold of the enzyme consists almost entirely of parallel β–strands wound into a right-handed β-helix. There are twelve turns in the β-helix, each comprising from one to three β-strands, leaving one side of the molecule with all the loop regions. A large groove is found at this side, which could be the possible substrate binding site. The molecule is highly glycosylated, with two N-glycosylation sites and 18 O-glycosylation sites. All O-glycosylation sites are located in the C-terminal tail of the molecule (367 - 422), which is a long random coil element, that surrounds the molecule. The O-glycosylation seems to protect the long C-terminal tail from proteolytic degradation and its function is probably to keep the otherwise hydrophobic molecule in solution. Two other plant cell wall degrading enzymes Pel C and Pel E are known and though they share the same three dimensional fold as rhamnogalacturonase A, they are slightly smaller with about eight turns in the right-handed β-helix.

**MS04.11.08 LIGHT AT THE END OF A 50Å LONG TUNNEL: CRYSTAL STRUCTURES OF ENZYME-OLIGOSACCHARIDE COMPLEXES REVEAL HOW CELLOBIOHYDROLASE I BINDS CELLULOSE.** Christina Divne, Jerry Ståhlberg & T. Alwyn Jones, Department of Molecular Biology, Biomedical Centre, Uppsala University, Box 590, S-751 24 Uppsala, Sweden.

We have previously reported the crystal structure of the major cellulase produced by the cellulolytic fungus *Trichoderma reesei*, cellobiohydrolase I (CBH I), in complex with a small saccharide ligand (1). CBH I is a retaining exo-cellulase that processively hydrolyzes alternating β-1,4-linkages of a cellulose chain from its reducing end (1) to liberate β-D-cellobiose as the main product. The active site is located near one end of a 50 Å long saccharide-binding tunnel and, hence, the cellulose chain has to be threaded through the tunnel prior to hydrolysis. Three carboxylate residues (E212, D214 and E217) were proposed to be involved in catalysis (1). These residues have been changed to their isosteric amide counterparts by means of site-directed mutagenesis (2) and all three mutations seriously impair the catalytic capability of the enzyme. Crystal structures of the mutant proteins in the absence of ligand have shown that no structural changes occur in their active sites (2). Crystal structures of the catalytically most deficient mutants, E212Q and E217Q, have now been successfully determined in the presence of cellotetraose, cellopentaose and cellohexaose at an effective resolution of 1.9 Å or better (3). For the first time, the complete mapping of glucose-binding sites in the tunnel of CBH I will be presented and the implications of the results for cellulose binding and catalysis will be discussed.

(1) Divne, C. *et al.* & Jones, A. (1994). *Science* **265**, 524-528.
(2) Ståhlberg, J. *et al.* & Jones, T. A. (1996). To be published.
(3) Divne, C. *et al.* & Jones, T. A. (1996). To be published.

**MS04.11.09 THE ROLES OF KEY RESIDUES IN THE 4/7 SUPERFAMILY OF GLYCOSYL HYDROLASES REVEALED BY CELLULASE:SUBSTRATE COMPLEX.** Joshua Sakon[1], Steven Thomas[2], Michael Himmel[2] and P. Andrew Karplus[1], Section of Biochemistry, Molecular and Cell Biology, Cornell University,Ithaca, NY 14853[1], National Renewable Energy Labs, Golden, CO 80401[2]

Cellulase E1 from Acidothermus cellulolyticus is a member of a large superfamily of β-glycosyl hydrolases characterized by a retaining mechanism and $(\alpha/\beta)_8$-barrel fold with three invariant active site residues: an adjacent Asn-Glu pair at the end of β- strand 4, in which the Glu serves as the acid/base in catalysis, and a nucleophilic Glu residue at the end of β-strand 7. The superfamily, encompasses families 1 (β-glucosidase, lactase phlorizin hydrolase, 6-phospho β-glucosidase, 6-phospho β- galactosidase, β-galactosidase, cyanogenic β-glucosidase), 2 (β- galactosidase, β-glucuronidase), 5 (cellulase, β-mannase), 10 (xylanase), 17 (β-1,3-1,4-glucanase), 30 (glucocerebrosidase), 35 (β-galactosidase), 39 (α-L-Iduronidase) and 42 (β-galactosidase). The crystal structure of the catalytic domain of E 1 complexed to cellotetraose solved by multiple isomorphous replacement method and refined to 2.4 Å resolution (R=17.9 % and $R_{free}$=23.8%), reveals the functional interactions made by the three known conserved residues: the nucleophilic glutamate is poised to attack the anomeric carbon; the

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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