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.
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**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