MS03 P15

Analysis of Carbohydrate Binding in a Family 5 Exoglucanase. John Cutfield, Yoshio Nakatani, Wayne Patrick, Sue Cutfield. *Biochemistry Department, University of Otago, Dunedin, New Zealand.* E-mail: john.cutfield@stonebow.otago.ac.nz

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The exo-ß-1,3-glucanase from *Candida albicans* (Exg) functions in cell wall remodelling through its glucosyl hydrolase and transferase activities. As with other family 5 glycosyl hydrolases Exg possesses catalytic glutamate residues acting as nucleophile (E292) and proton donor (E192). The entrance to the active site pocket is flanked by a pair of antiparallel phenylalanine residues (F144 and F258). Crystallographic analysis of enzyme:inhibitor complexes has identified protein-sugar interactions at the -1 site and, to a lesser extent, at the +1/+2 aromatic entranceway [1]. We have made various mutants to try and better identify sugar-binding sites, to alter the ratio of hydrolysis to transfer and to compare substrate specificity for hydrolase and glucosynthase reactions.
Mutations at the Phe-Phe gateway demonstrated that aromaticity must be preserved and that the transferase reaction was more sensitive to mutation, consistent with two docking events. The catalytically disabled mutant E292Q did not show coherent electron density to indicate stable binding of oligosaccharide substrates but unexpectedly revealed an external glucose binding site that may provide a foothold for the yeast cell wall D-glucan. Exg was converted to a glucosynthase via E292S and crystallized in the presence of donor 1-fluoro-α-D-glucose and acceptor p-nitrophenyl-ß-D-glucopyranoside [2]. The two sugars were oriented such that donor sugar C1 was close to acceptor O6, consistent with nmr analysis of solution products which showed the main product to be ß-1,6-linked disaccharide and not ß-1,3- as expected.

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[2] Williams SJ, Withers SG, *Carbohydr. Res*. 2000, 327, 27.

MS03 P16

Crystallographic and kinetic studies of rhamno-galacturonan lyase from *Aspergillus aculeatus*

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Rhamnogalacturonan lyase (AA-RGL) from *A. aculeatus* is a pectin backbone degrading enzyme that belongs to the polysaccharide lyase family 4 [1]. The structure of AA-RGL [2] was previously determined at a resolution of 1.5 Å. The enzyme is comprised of three domains. The domains display structural homology with domains from other carbohydrate active enzymes, but neither of these domains possess any catalytic activity. Based on sequence alignments within polysaccharide lyase family 4 the location of the active site was proposed and possible candidates for the catalytic base and other active residues were identified [2]. Two of these putative active site residues were mutated (K150A and H210A). Preliminary kinetic studies have shown that the two mutants of AA-RGL have low catalytic activity. The two mutant enzymes could be crystallized in conditions similar to those of the native enzyme, 20% PEG 4000, 0.1 M $(NH_4)_2SO_4$ and 0.1 M sodium acetate pH 4.3. Beautiful single plate-like crystals of both mutants were obtained by seeding from plate clusters of the H210A mutant. The crystals of both mutants diffract to a resolution better than 1.9Å on a in house equipment. The diffraction data revealed a tetragonal space group, $P4_32_12$, like the native enzyme. Cell dimensions were a = b = 77.0 Å and c = 170 Å. Preliminary structure determination show that the mutations do not disturb the overall structure, consistent with a direct role of K150 and H210 in catalysis or substrate binding.

[1]. Coutinho PM & Henrissat B. (1999) Carbohydrate-Active Enzymes server at URL: http://afmb.cnrs-mrs.fr/CAZY/
[2]. McDonough MA, Kadirvelraj R, Harris P, Poulsen JC, Larsen S. (2004), *FEBS Lett*. **565**, 188-94

MS03 P17

Structure of the full-length response regulator PleD in its activated state Tilman Schirmer,Paul Wassmann, Dietrich Samoray, *Biozentrum, University of Basel, Switzerland.* E-mail: tilman.schirmer@unibas.ch

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During the last decade cyclic di-GMP has become recognized as a novel ubiquitous second messenger in eubacteria [1]. Condensation of two GTP to the dinucleotide is catalyzed by two identical diguanylate cyclase domains (DGC or GGDEF) occurring in various combinations with sensory and regulatory modules. One of these modular proteins is PleD – a response regulator receiver composed of two CheY like domains and a DGC domain [2], thus merging "two-component" signal transduction with c-diGMP signaling. PleD is involved in the cell morphology development of the marine bacteria *Caulobacter crescentus*, namely conversion of the motile (flagelled) to the sessile (stalked) form [3].
Like other response regulators, PleD is activated by a cognate histidine kinase through phosphorylation at Asp53 of the first CheY-like domain. Since phospho-aspartates are short-lived, structure analysis of the activated state is challenging. The problem was overcome by chemical modification with BeF_3^-, which was previously shown for CheY to act as a phosphate analog[4]. Such activated PleD was crystallized in a new crystal form showing the modified Asp affecting adjacent residues e.g. Thr85, Phe102 and causing large changes in the β4-α4 loop as seen before for response regulator receivers [4]. These movements result in a relative rearrangement of the CheY-like domains of a PleD monomer, which allows the formation of a tight dimer, bringing the two DGC domains in near proximity to each other. Such a dimer is the basis for the catalytic activity of PleD, namely formation of c-diGMP.
PleD shows non-competitive product inhibition. An allosteric c-diGMP binding site between the DGC-domain and the second CheY-like domain had been identified