Acta Cryst. (2002). A**58** (Supplement), C238

RHAMNOGALACTURONAN LYASE REVEALS A UNIQUE FOLD AND SUGGESTS A COMMON POLYSACCHARIDE LYASE MECHANISM

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Rhamnogalacturonan (RG), known as the hairy portion of the pectin network of the primary plant cell wall, is composed of repeating dimeric units of -(1-2)-αa-L-rhamnopyranosyl-(1,4)-αa-D-galactopyranosyluronide- with varying degrees of branching of Rha residues and acetyl or methyl esterification of GalUA residues. Rhamnogalacturonan lyase (RG-lyase) cleaves the α 1-4 glycosidic bond of the RG polysaccharide backbone leaving an α-Δ-(4,5)-unsaturated D-GalUA at the non-reducing end and L-Rha at the reducing end. A unique feature of this enzyme is its requirement for very large substrates. We have determined the structure of RG-lyase from *Aspergillus aculeatus* by MIR and refined it to 1.5 Å resolution. This is the first known structure of a CAZy Family 4 polysaccharide lyase. RG-lyase exists as a monomer with 508 residues (M.W. = 54.2 kDa). A three-domain structure made up of mostly β - sheet with an overall shape of a flattened rugby ball is observed. No closely homologous structures have been found using the DALI server suggesting that RG-lyase has an overall unique structural fold. The three intimately interacting domains consist of a 251 residue N-terminal domain, an 86 residue mid domain, and the remaining 171 residues make up the C-terminal domain. The proposed active site is situated in the deepest pocket on the surface of the enzyme within a groove made up from all three domains that is large enough to accommodate the polysaccharide substrate. It is proposed that the catalytic machinery of RG-lyase is similar to that of other polysaccharide lyases.

Keywords: RHAMNOGALACTURONAN, LYASE, PLANT CELL WALL

Acta Cryst. (2002). A**58** (Supplement), C238

THE STRUCTURE OF THE ORANGE CAROTENOID PROTEIN

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Carotenoid-binding proteins function in light-harvesting and in photoprotection by quenching reactive-oxygen species. The structure of the orange carotenoid-binding protein (OCP) isolated from the cyanobacterium Arthrospira maxima has been determined at a resolution of 2.0 Å by multi-wavelength anomalous dispersion methods. OCP is the first structural example of a protein that binds exclusively carotenoids. The primary structure of OCP has no close homologs and contains an internal sequence duplication. The structure of OCP is a novel composite of three domains; all three are involved in shielding the carotenoid from solvent. A proteolytic product of OCP, isolated in the laboratory, appears red instead of orange. Comparison of the primary structure of the red fragment of the protein with OCP structure provides specific detail about how the protein tunes the carotenoid's absorption properties. Details of the interaction between the pigment and protein will also be discussed in the context of OCP's putative function in photoprotection.

Keywords: NOVEL FOLD, PIGMENT PROTEIN, PHOTOSYNTHESIS

Acta Cryst. (2002). A**58** (Supplement), C238

STRUCTURES OF PLATELET-RECEPTOR GLYCOPROTEIN Ib AND ITS COMPLEX WITH VON WILLEBRAND FACTOR DOMAIN A1

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Transient interactions of platelet-receptor glycoprotein Ibα (GpIbα) and immobilized von Willebrand Factor (vWF) mediate rolling of blood platelets at sites of vascular damage. This phenomenon initiates platelet adhesion and is essential for haemostasis in rapidly flowing blood. In the circulation GpIbα and vWF coexist, but do not interact at a detectable level unless shear stress is applied. Shear-induced interaction in occluded atherosclerotic arteries contributes critically to the onset of thrombosis. We have determined structures of the GpIbα N-terminal domain (residues 1-290) and its complex with vWF domain A1 (residues 498-705) at resolutions of 1.8 and 3.1 Å, respectively. Crystallisation of the complex required the use of mutants GpIbα -M239V and A1-R543Q that enhance complex formation and are associated with platelet-type and type 2B von Willebrand diseases. The N-terminal domain of GpIbα displays an elongated curved shape formed by eight leucine-rich repeats flanked by conserved capping sequences. In the complex, GpIbα wraps around one side of A1 providing two contact areas bridged by an area of solvated charge interaction. Mutations related to platelet-type von Willebrand disease induce β-hairpin formation in a flexible loop of GpIbα. The β-hairpin provides a key interaction in the complex by forming an inter-molecular β-sheet. Mutations related to type 2B von Willebrand disease destabilize the conformation of the N- and C-terminal peptides of A1 that may otherwise shield the GpIbα binding-site. This mechanism possibly mimics shear-induced activation. Thus, our data provide detailed insights into the initial interactions in platelet adhesion that are relevant for the development of anti-thrombotics.

Keywords: LEUCINE-RICH REPEAT CELL ADHESION HAEMOSTASIS

Acta Cryst. (2002). A**58** (Supplement), C238

THE *DE NOVO* PHASING OF NATIVE MACROMOLECULAR STRUCTURES USING SOFTER X-RAYS

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The greater accessibility of synchrotron beam-lines for macromolecular crystallography has resulted in a broad wavelength range now being available for use in the collection of X-ray diffraction data from single crystals of macromolecules. The softer (long wavelength) end of this spectrum ($\lambda > 1.5$ Å) has recently become attractive as it allows routine phase determination in the de novo elucidation of native crystal structures by targeting the anomalous scattering properties of sulfur and/or phosphorous. Although the anomalous signals available at routinely accessible wavelengths (1.8 Å) are rather small, a test-case study on two crystal forms of Tryparedoxin II from *Crithidia fasciculata* has shown that they are both measurable and useable for structure determination even from crystals that diffract to medium resolution. This method of structure solution has enormous potential in the era of structural genomics and, as will be seen in this presentation, it should soon become a major weapon in the armory of macromolecular crystallographers.

Keywords: ANOMALOUS DISPERSION SOFT X-RAYS MACROMOLECULAR STRUCTURE