Sedimentation equilibrium studies were conducted using bFGF and a number of heparin analogs. The results have shown that several different heparin analogs do indeed cause association of bFGF in solution. Unliganded bFGF was run as a control in each experiment, and no association was seen. These studies are being continued in order to further characterize the nature of the ligand-induced association caused by an active nonsulfated triasaccharide (Tri-3), an inactive sulfated disaccharide (sucrose octasulfate), and a sulfated heparin octasaccharide fragment. These ultracentrifugation studies provide support for the model of ligand-induced association of bFGF as suggested by the co-crystal structures.


The mannos transporter from *Escherichia coli* is a member of the phosphoenolpyruvate-dependent phosphotransferase system (PTS). The multi-subunit complex couples translocation across the bacterial inner membrane with solute phosphorylation. A functional fragment, 14.8 kDa (IABMan, residues 2-133) of the membrane associated IABMan subunit of the mannos transporter was expressed as a selenomethionine protein and the unphosphorylated structure was solved by X-ray crystallography. The protein consists of a central 5-stranded β-sheet flanked by helices. The order of the secondary structure elements is (β0)4, (α2)4 with strand order 21345. Four parallel β-strands are linked by helices forming right-handed cross-over connections. The fifth strand is antiparallel to the others and is swapped between the subunits of the dimeric structure. Helices D and E form a helical hairpin. The active site consists of a buried aspartyl group and a histidine residue which is reminiscent of the family of serine proteases. His10, which is known to be transiently phosphorylated during catalysis, is located at the topological switch-point of the structure and close to the subunit interface. N81 of His10 is hydrogen bonded to the sidechain of Asp67. It is likely that Asp67 acts as a general base and thus increases the nucleophility of the histidine. Data were collected to 1.6 Å at station 9.5 in Daresbury and the crystal structure was solved in a hexagonal spacegroup P61(122) (Imol/a.u.) with cell constants a=b=76.4 Å, c=88.7 Å, α=β=90°, γ=120° using MIRAS. The initial Se-SIRAS map calculated to 2.4 Å showed part of a helix (later identified as helix D) which improved upon addition of the platinum phases (calculated to 3.2 Å) and density modification. The atomic model was built and refined to 1.7 Å resolution with a crystallographic R-factor of 18.9% and Rfree of 22.1% for 989 protein atoms and 70 water molecules.

**PS04.11.25 INTERACTION OF CHITOBIOSIDE WITH PNGASE F MUTANTS.** Peter Kuhn, SSSL, Stanford, CA 94309, USA and Patrick Van Roey, Wadsworth Center, NYSDOH, Albany, NY 12201, USA

Peptide-N4-(N-acetylgalcosaminyl)asparagine amide F (PNGase F) is an amidohydrolase secreted by *Flavobacterium meningosepticum*. The enzyme releases intact Asn-linked oligosaccharides from glycoproteins and glycopeptides while converting the asparagine residue to an aspartic acid. Based on crystallographic and mutagenesis studies, three acidic residues, Asp60, Glu118 and Glu206, were shown to be important for catalytic activity or substrate binding. All three residues are found in a cleft at the interface between the two β-sandwich domains of the enzyme. Asp60 and Glu206 are located at one end of the cleft and are connected by a bridging water molecule. Glu118 is located towards the other end of the cleft. The structures of the amide mutants of all three residues, Asn60, Gln118 and Gln206, are essentially identical to that of the wild-type enzyme. This proves that the reduced activities of the mutants, less than 0.01% of the wild-type enzyme, result from the chemical modification and not from a structural effect. The structures of the wild-type enzyme and all three mutants have also been determined using crystals that were co-crystallized with 30-fold excess of N,N'-diacetychitobiose, the Asn-proximal core of the oligosaccharide product. The structural analysis shows that the disaccharide binds well to the wild-type enzyme and to the Gln206 mutant, less well to the Asn60 mutant and not at all to the Gln118 mutant. These results are consistent with the location of the disaccharide in the binding site: O1 of the first GlcNAc forms a hydrogen bond with Asp60, while O6 of the second GlcNAc is in hydrogen bonding contact with Glu118. Therefore, Asp60 is most likely the primary catalytic residue, while Glu206 plays a secondary in catalytic process. Glu118 is essential for substrate binding but not involved in the catalytic mechanism.


Glycosaminoglycans (GAG’s) are polymeric saccharide structures found in the extracellular matrix, and on the cell surface of many cell types where they are linked to core proteins. Their biological function ranges from serving as a protective barrier to diffusion, to the modulation of cell signalling. In vivo, there are two main mechanism for their degradation: the eliminative cleavage by lyases and the hydrolytic cleavage by hydrolases. The enzymes responsible for this degradation are specific for particular sequences in the GAG chain. Degradation of GAG’s (or the lack thereof) has been linked to particular diseases, and the enzymes involved are potential targets for new pharmaceuticals. Chondroitinase AC represent one of the three categories of lyases; it is responsible for the cleavage of chondroitin-4-sulfate, and chondroitin-6-sulfate. In order to understand the structural aspect of the narrow substrate specificity of these lyases, we have undertaken the structure determination of chondroitinase AC from *Flavobacterium heparinum*. The enzyme expressed in *E. coli* is a monomer of 77 kDa with two O-glycosylation sites. Tetragonal crystals of 0.2 x 0.2 x 1.0 mm are obtained after 3 weeks from a solution containing PEG 3350. They belong to the space group P43212 or its enantiomorph P43212. The unit cell dimensions are a=b=87.2 Å, and c=192.8 Å. Assuming one 77 kDa molecule in the asymmetric unit, one obtains a Matthews coefficient of 2.37, a typical value for protein crystals. The crystals are sensitive to radiation damage, thus they were flash frozen for data collection. A 2.9 Å dataset was collected at 130K. We are now in the process of screening for heavy atom derivatives in order to solve the structure by the multiple isomorphous replacement method. Initial screening identified potential derivatives that will be used for the structure determination.

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