a calcium binding site which is essential for catalytic activity. The third structural domain is only loosely attached to the core of the enzyme and its functional role remains unclear. These structural studies have allowed for a detailed mapping of catalytic elements directly in the active site region and associated residues within the nearby elongated surface substrate binding groove. Further work has focused on determining the structure of the complex formed by the clinically utilized inhibitor acarbose and human pancreatic α-amylase. This work has shown this enzyme catalyzes a rearrangement of the individual components of acarbose to produce a stable enzyme-product complex. An important part of this process involves a concerted movement of a surface polypeptide chain loop to closely interact with the bound inhibitor. On the basis of these results attempts are in progress to develop novel high affinity inhibitors of human α-amylase. The goal of this work is not only to provide additional insight into the catalytic mechanism of this enzyme, but also to identify potential enhanced therapeutic agents.

PS04.11.21 IMPORTANCE OF GLUTAMIC ACID 35-TRYPTOPHAN 109 CONTACT IN THE MAINTENANCE OF THE CATALYTIC CLEFT GEOMETRY OF HUMAN LYSOZYME. M. Muraki, S. Goda, H. Nagahora and K. Harata, National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki 305, Japan

Human lysozyme catalyses the hydrolysis of glycosidic linkages in the peptidoglycan of the bacterial cell wall. In the crystal structure of wild-type human lysozyme, the side-chain of Glu35 that acts as a general acid in the catalytic action and the side-chain of Trp109 are located very close to each other within the distance of van der Waals contact. Mutations of human lysozyme replacing Glu35 with Asp or Trp109 with Phe reduced the lytic activity against *M.luteus* cells to ca. 0.3% and ca. 20% of that of wild-type enzyme, respectively. In order to investigate the structural effect of these mutations and to clarify the cause of the functional change, the structures of the mutant enzymes were analysed by X-ray crystallography. The refinements were carried out at 1.66 Å resolution (Glu35Asp mutant) and at 1.60 Å resolution (Trp109Phe mutant). The coordinate errors were estimated to be 0.16 Å-0.17 Å for either mutant. The RMS values of the distance between the corresponding α-carbon atoms of the mutant enzyme and that of wild-type enzyme were 0.12 Å (Glu35Asp mutant) and 0.13 Å (Trp109Phe mutant), indicating no significant change in the global conformation of the molecule. However, a remarkable change in the local conformation was detected in either mutant as compared with wild-type enzyme. The difference of 0.4 Å-0.7 Å in the distance between the corresponding α-carbon atoms from that of wild-type enzyme were found in the region from residue 110 to 118 (Glu35Asp mutant) and from residue 104 to 111 (Trp109Phe mutant). The above result together with the results of kinetic analysis suggests the existence of the contact between Glu35 and Trp109 is important not only in making the hydrophobic environment around the carboxylate group of Glu35 but also in the maintenance of the catalytic cleft geometry, which is responsible for the formation of the "productive" complex between the enzyme and the substrate.

PS04.11.22 MIRAS PHASING OF THE CRYSTAL STRUCTURE OF THE GLYCOGEN DEBRANCHING ENZYME PROM MAMMALIAN MUSCLE. J. P. W. Petersen, E. M. Bergmann, N. B. Madsen, M. N. G. James, MRC Group in Protein Structure and Function, Dept. of Biochemistry, U of Alberta, Canada TOG 2H7

Glycogen debranching enzyme from rabbit muscle (GLIX) is a monomeric enzyme consisting of 1555 amino acids with a molecular weight of 178 KDa [1]. GLIX removes α-1,6 branch points in the limit dextrin left by the action of phosphorylase during the physiological degradation of glycogen. The removal of the α-1,6 branch points involves two different enzymatic activities. A glycosyl transferase activity which moves three glucose units from the side chain to the main chain and an α-1,6 glucosidase activity which removes the remaining α-1,6 linked glucose unit. Both activities are present in a single large subunit.

Crystals of GLIX belong to space group $P2_12_12_1$ with unit cell dimensions a=105.0Å, b=195.0Å & c= 92.5Å and has one molecule per asymmetric unit [2]. A native data set was collected to 2.8Å. Furthermore, data were obtained for more than a dozen heavy atom derivative crystals. Currently we have obtained four useful derivative data sets: methylmercurychloride (1 and 2 sites respectively), KUO_2F_5 (1 site) and goldthioglucose (2 sites). The best derivative (methylmercurychloride) is isomorphous to 3.5Å (R_{iso}=15.0 %)

Good MIRAS phases to 4.5Å allowed us to identify the solvent regions and domain structure of the crystal. Three of the derivatives have the major site in common. The only unique derivative (uranium) indicates non-isomorphism beyond 4Å resolution. Heavy atom phases to 3.4Å resolution are available and will be improved by various methods including solvent flattening, histogram matching, iterative skeletonization and envelope editing.The poster will present the current status of the structure determination of GLIX.

[1] Liu *et al.* Arch. Biochem. Biophys. 306, 232 (1993)
[2] Fitzgerald & Madsen. J. Crystal Growth. 76, 557 (1986)

PS04.11.23 CO-CRYSTAL STRUCTURES OF BASIC FIBROBLAST GROWTH FACTOR COMPLEXED WITH DI- AND TRISACCHARIDE HEPARIN ANALOGS. Andrew B. Herr, David M. Ornitz*, and Gabriel Waksman, Department of Biochemistry and Molecular Biophysics and *Department of Molecular Biology and Pharmacology, Washington University Medical School, St. Louis, MO 63110, USA

Basic fibroblast growth factor (bFGF) has been co-crystallized with two different nonsulfated heparin-like oligosaccharides, providing the first view of the binding sites of biologically active heparin analogs (Ornitz, Herr, et al., Science 268:432, 1995). Both oligosaccharides are nonsulfated, isomerically pure analogs that show significant biological activity. The co-crystal structures revealed three saccharide binding sites per bFGF monomer, designated sites 1, 2, and 2'. The co-crystals of each bFGF/saccharide complex show identical binding sites. Site 1 is identical to the previously proposed high-affinity heparin binding site. Binding sites 2 and 2', however, have not been previously identified. These two sites occur at a crystal packing interface between two neighboring bFGF monomers, and a single saccharide ligand bridges the two binding sites. Analysis of the number of hydrogen bonds between the ligand and each site indicates that each is an independent binding site and not merely an artifact of crystal packing. The observation of a single ligand bridging two binding sites in adjacent bFGF molecules suggests that these ligands may induce bFGF self-association.

Analytical ultracentrifugation was used to test whether small heparin analogs can induce self-association of bFGF in solution.

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 trisaccharide (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.

PS04.11.24 THE CRYSTAL STRUCTURE OF THE IIA DOMAIN OF THE MANNOSE TRANSPORTER FROM *ESCHERICHIA COLI* AT 1.7 Å RESOLUTION. R. S. Nunn[1], Z. Markovic-Housley[1,2], J.C. Genovesio-Taverne[1], K. Flukiger[2], P.J. Rizkallah[3], J.N. Jansonius[1], B. Erni[2] and T. Schirmer[1], [1]Biozentrum, Abteilung für Strukturbiologie, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland, [2]Institut fur Biochemie, Universitat Bern, Freiestrasse 3, CH-3012 Bern, Switzerland. [3]Daresbury Laboratory,Daresbury, Warrington,WA4 4AD, UK

The mannose 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 (IIAMan, residues 2-133) of the membrane associated IIABMan subunit of the mannose 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 ß-sheet flanked by helices. The order of the secondary structure elements is (ßα)4,(αß) with strand order 21345. Four parallel ß-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 topologial switch-point of the structure and close to the subunit interface. Nδ1 of Hisl0 is hydrogen bonded to the side-chain of Asp67. It is likely that Asp67 acts as a general base and thus increases the nucleophilicity 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 P6(1)22 (Imol/a.u.) with cell constants a=b=76.4 Å, c=88.7 Å, α=ß=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 Rfactor of 18.9% and Rfree of 22.1% for 989 protein atoms and 70 water molecules.

PS04.11.25 INTERACTION OF CHITOBIOSE WITH PNGASE F MUTANTS. Peter Kuhn, SSRL, Stanford, CA 94309, USA and Patrick Van Roey, Wadsworth Center, NYSDOH, Albany, NY 122201, USA

Peptide-N4-(N-acetylglucosaminyl)asparagine amidase 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'-diacetylchitobiose, 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..

PS04.11.26 PRELIMINARY CRYSTALLOGRAPHIC ANALYSIS OF CHONDROITINASE AC FROM *FLAVOBACTERIUM HEPARINUM*. J. Féthière, B. H. Shilton, Y. Li, M. Laliberté, B. Eggimann, M. Cygler, Biotechnology Research Institute, National Research Council of Canada, Montréal, Qué. Can., H4P 2R2, IBEX Technologies, Montréal, Qué. Can. H4P 1P7

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 $P4_32_12$ or its enantiomorph $P4_12_12$. The unit cell dimensions are a=b=87.2Å, and c=192.8Å. Assuming one 77 kDa molecule in the assymetric 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 2.9Å 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.