PS04.11.17 CRYSTALLOGRAPHIC STUDIES ON MALTOTETRAOSE-FORMING AMYLASE FROM ALCALIGENES SP. Jian-Hua Ma, Zi-Zheng Yan#, Yao-Ping Wang, Yi Han, Ru-Chang Bi. Institute of Biophysics, Academia Sinica, Beijing 100101, P.R.China; #Institute of Microbiology, Academia Sinica, Shanghai 100080, P.R.China

Preliminary crystallographic study has been carried out with maltotetraose- forming amylase(E.C.3.2.1.60, G4-amylase), which was isolated and purified from Alcaligenes sp. found from Chinese soil. G4-amylase is a unique amylase which catalyzes the release of α-maltotetraose from the nonreducing ends of starch molecules. It is commercially important for producing maltotetraose with superior properties. This enzyme has molecule mass of about 60kD and pI4.45.

After an intensive screening of crystallization conditions was conducted with the enzyme, better G4-amylase crystals could be obtained using the hanging drop method with a drop consisting of 10mg/ml enzyme sample solution and equal volume of reservoir solution containing 0.1M Cacodylate buffer (pH6.5), 0.2M calcium acetate and 18% PEG-8000.

The G4-amylase crystals are orthogonal, and the unit cell has dimensions a=46.6Å, b=65.8Å and c=170.9Å and one molecule per asymmetric unit. 2.8Å intensity data have been collected with a G4-amylase crystal on Mar Research IP detector system in our laboratory. Further structure determination of G4-amylase is under way.

PS04.11.18 PRELIMINARY STRUCTURE CHARACTERIZATION OF HUMAN ACID β-GLUCOSIDASE. Feng Luo and Hengming Ke, Department of Biochemistry & Biophysics, School of Medicine, University of North Carolina, Chapel Hill 27599-7260, USA

Acid β-glucosidase is an enzyme which hydrolyzes glucosylceramide to ceramide and glucose. Deficiency activity of the enzyme causes the accumulation of glucosylceramide in tissues, leading to a prevalent lysosomal storage disease known as Gaucher disease. The enzyme replacement therapy is the most efficient treatment of Gaucher disease, however, it is limited by its cost, at an estimate of 765,000 per patient per year. The *Wall Street Journal* and the *New York Times* called it the world most expense drug.

Our study aims at crystallization and structural characterization of the native acid β-glucosidase, its complexes with the competitive inhibitor of N-butyl deoxynojirimycin and the transition state analogue of 2-fluoro-2-deoxyglucoside fluoride. These crystal structures will provide insight into the catalytic mechanism and help with screening the most efficient mutant for the new therapy of gene transfer. N-butyl deoxynojirimycin is specially interesting because it has been reported to inhibit the infectivity of HIV-1 and SIVmac and is currently in clinical trials for AIDS.

Crystal with a dimension 0.1 mm x 0.1 mm x 0.2 mm of the native acid β-glucosidase has been obtained by the vapor diffusion method in the following conditions: protein concentration of 3 mg/ml, protein buffer (50 mM malaic acid, 4 mM β-mercaptorethanol, 0.75 M ammonium phosphate, 1% glycerol, pH 6.5), reservoir buffer (50 mM malaic acid, 4 mM β-mercaptorethanol, 1.2 M ammonium phosphate, 1% glycerol, pH 6.5), and the protein drop (3 μl of the protein buffer and 3 μl of the storage protein solution).

PS04.11.19 STRUCTURAL IMPLICATION OF TWO SACCHARIDE CONFORMATIONS IN THE ACTIVE SITE OF A ß-GLYCOSIDASE A. White*, D. Tull§, K. L. Johns*, S. G. Withers§, and D. R. Rose*; *Department of Medical Biophysics, University of Toronto and Ontario Cancer Institute, Toronto, Canada M5G 2M9; §Department of Chemistry, University of British Columbia, Vancouver, Canada V6T 1Z1.

It is well established from biochemical and structural studies that a saccharide deformation takes place during catalysis by glycosyl hydrolases. In the context of two catalytic carboxylates in the active site of most of the retaining ß-1,4-glycosidases, the hydrolysis is believed to proceed by a double displacement catalytic mechanism through a covalent intermediate with oxocarbenium transition states. Our previous crystallographic studies revealed that a covalent α-glycosyl-enzyme catalytic intermediate can be accommodated in the confined space of the active site of the enzyme cex-cd. [White, A., *et al.*, 1996, *Nature Struct. Biol.* 3:149] In this structure the attached saccharide adopts a chair conformation which differs from the planar arrangement of the transition states. We report here the structure of cex-cd complexed with the inhibitor cellobial (1,2-ene-1,2-dideoxy-cellobiose) designed to mimic the sugar conformation of the transition states.

Soaking of a crystal of the bacterial xylanase/cellulase cex-cd in 20 mM cellobial induces less than 0.2% change to the $P4_12_12$ unit cell parameters. Data to 2.2 Å resolution were collected using a SDMW area detector and then reduced to an R-merge of 0.07 . The crystal structure of the unliganded cex-cd [White, A., *et al.*, 1994, *Biochemistry* **33**:12546] was used to solve its complexed form. At the current stage of refinement with X-PLOR the R-factor is 0.175 with a free R value of 0.26, given a data to parameters ratio of 1.4 and good model stereochemistry. The difference Fourier electron density map reveals a prominent element of electron density in the active site of cex-cd, indicating the presence of a cellobial molecule. Compared to the fluorocellobiosyl covalent complex [White *et al.*, 1996], the cellobial occupies the same subsites and makes a similar network of interactions between the distal saccharide and the enzyme. Further comparison of the structure of these liganded forms of cex-cd may inform on the catalytic mechanism of retaining ß-glycosidases.

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PS04.11.20 INHIBITOR REARRANGEMENT FOLLOWING COMPLEX FORMATION BETWEEN ACARBOSE AND HUMAN PANCREATIC α-AMYLASE. Yaoguang Luo, Stephen G. Withers, Chris M. Overall and Gary D. Brayer, Departments of Biochemistry and Molecular Biology, Chemistry, and Clinical Dental Science, University of British Columbia, Vancouver, B. C. Canada V6T 1Z3.

The complex sugar starch forms the principal source of glucose in the human diet. Initial starch digestion is provided by a salivary α-amylase, and then upon reaching the gut these degradation products are more extensively hydrolyzed by an α-amylase secreted by the pancreas. The salivary and pancreatic α-amylases are closely related isozymes which are expressed in a tissue-specific manner. Each of these enzymes are composed of a single polypeptide chain (MW=55,000) consisting of 496 amino acids. To gain a comprehensive understanding of the catalytic mechanism of human pancreatic α-amylase, we have completed the 1.8Å structure of this enzyme using x-ray diffraction techniques. These studies show this enzyme is composed of three structural domains. The core of the most prominent of these consists of an 8-stranded parallel β-barrel surrounded by extensive α-helical segments. To one end of this domain is located the active site region and a chloride binding site. A second domain is constructed around

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 FROM 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.