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