PS04.11.17 CRYSTALLOGRAPHIC STUDIES ON MALLOTTETROASE-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 (EC 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 pH 4.45.

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 10ng/ml enzyme sample solution and equal volume of reservoir solution containing 0.1M cacodylate buffer (pH6.5), 0.2M calcium acetate and 1% 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 CHARACTORIZATION OF HUMANACID 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 the enzyme causes the accumulation of glucosylceramide in tissues, with the competitive inhibitor of N-butyl deoxynojirimycin and specially interesting because it has been reported to inhibit the enzyme with the competitive inhibitor of N-butyl deoxynojirimycin and 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 P41212 unit cell parameters. Data to 2.2 Å resolution were collected using a SDM1 area detector and then reduced to an R-value of 0.07.

The crystal structure of the unliganded cex-cd [White, A., et al., 1994, Biochemistry 33:12546] was used to solve its complex form. At the current stage of refinement with x-repo 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 β-glycosidases. Supported by the Protein Engineering Network of Centres of Excellence.

PS04.11.19 STRUCTURAL IMPLICATION OF TWO SACCHARIDE CONFORMATIONS IN THE ACTIVE SITE OF A β-GLUCOSIDASE. A. White*, D. Tulli, K. L. Johns*, S. G. Withers*, and D. R. Roe; "Department of Medical Biophysics, University of Toronto and Ontario Cancer Institute, Toronto, Canada MSG 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 β-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.

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...