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.