02.1-23 CRYSTAL STRUCTURE OF β-LACTAMASE FROM *STAPHYLOCOCCUS AUREUS* PC1 AT 2.5 Å RESOLUTION. By Osnat Herzberg and John Moult, Medical Research Council of Canada Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Canada T6G 2H7.

β-lactamases are enzymes which bacteria have acquired as a defence against β-lactam antibiotics. The crystal structure of a class A β-lactamase from *Staphylococcus aureus* PC1 has been determined at 3.0 Å resolution, and refined at 2.5 Å resolution. The structure determination employed three rather low quality heavy atom derivatives, and solvent flattening of the resulting electron density map. The molecule consists of two closely associated domains with a novel folding topology. One domain is formed by a five stranded antiparallel β-sheet, and three helices that pack against one face of the sheet. The second domain packs against the second face of the sheet, and is mostly helical. The completely buried nature of the β-sheet is unusual for an antiparallel structure. The helical domain can be regarded as an insert in the middle of the sheet containing domain, such that the latter domain is formed by the N- and C-termini of the sequence. In the helical domain, a central helix is surrounded by five other helices. The active site serine, Ser 70, is located at the N-terminus of this helix, at the bottom of a depression formed at the interface between the two domains. Examination of the disposition of the functionally important residues, has led to a model for the binding of a substrate. There is a functional analogy to the serine proteases, involving an oxyanion hole and a lysine residue in an equivalent position to that of the serine protease histidine. Questions concerning the evolutionary relationship to the β-lactam target enzymes of the bacterial cell wall are addressed by comparing the folding of one of these enzymes and that of β-lactamase.

02.1-24 THE CRYSTAL STRUCTURE ANALYSIS OF XYLANASE FROM *Bacillus pumilus* IPO . By Yasuo Hata, Hiroshi Yamaguchi, Hideaki Moriyama*, Mamoru Sato, Nobuo Tanaka, Hirosuke Okada* and Yukiteru Katsube, Institute for Protein Research, *Department of Fermentation Technology, Faculty of Engineerig, Osaka University, Suita, Osaka 565, Japan

Xylanase from *Bacillus pumilus* IPO is an extracellular endo-enzyme which hydrolyzes β-1,4-xylan to xylo-oligosaccharide. The molecule is a single polypeptide chain consisting of 201 amino acid residues (Mr=22,500). The amino acid sequence was deduced from the nucleotide sequence. The crystals were obtained from PEG 6000 solution by microdialysis method. They were monoclinic with space group $P2_1$ and unit cell dimensions of a=40.8, b=66.8, c=34.7 A and β=103.1°. There was one molecule per asymmetric unit. Two heavy atom derivatives (K_2PtCl_6 and $UO_2(CH_3COO)_2$) were prepared by soaking. A 2.9 A electron density map calculated with double isomorphous replacement was tentatively interpreted. The molecule has an ellipsoidal shape with approximate dimensions of 40x35x30 A. A crevice (10 A in diameter) running through the molecule separates it into two domains. The active site may be in the vicinity of the crevice which is large enough to bind its substrate, a chain molecule of xylan. Such structural features of xylanase are similar to those of lysozyme. However, xylanase adopts the β-sheet structure which is different from that of lysozyme consisting mainly of α-helices. The analysis of the detailed structure is in progress.

02.1-25 REFINED STRUCTURES OF *D*-GALACTOSE BINDING PROTEIN FROM E. COLI AND ITS MUTANT REVEAL SUGAR, CALCIUM AND TRANSDUCER BINDING SITES. Nand K. Vyas, , Meenakshi N. Vyas, Guang Y. Lu, & Florante A. Quiocho, Howard Hughes Medical Institute, Baylor College of Medicine and Department of Biochemistry, Rice University, Houston, Texas, USA.

The structure of the native D-galactose binding protein (GBP), an initial receptor for active transport and chemotaxis in bacterial cells, has been determined at 1.9 Å resolution. The starting phases derived from recently published GBP model at 3.0 Å resolution were improved and extended to 1.9 Å resolution by combination of several phase extention and refinement methods. The restrained least squares refinement of the complete GBP structure reduced the R-factor to 0.15 for all well merged observable data between 10. to 1.9 Å resolution. The deviation from ideality in bond lengths and bond angles are of the order of 0.02 Å and 0.04 Å, respectively. The overall structure of GBP is very similar to three other binding protein structures (33K to 45K dalton) solved in our laboratory with specificity for L-arabinose, sulfate, and Leu/Ile/Val. All these binding proteins consist of a single polypeptide chain which folds into a two-domain structure. A deep substrate specific binding site cleft is formed between the two domains. Despite the overall structural similarity, binding proteins have no appreciable sequence homology. In the GBP structure, the bound D-glucose, also a natural substrate is held in place primarily by hydrogen bonds. The sugar binding residues and sugar itself are inaccessible to bulk solvent. Many features of the GBP-sugar interaction are similar to those originally discovered in the 1.7 Å resolution strucuture of the L-arabinose binding protein (Quiocho, F. A. and Vyas, N. K., 1984, *Nature*, **310**, 381-386). The attachment site in GBP for the membrane-bound chemotactic signal transducer (trg) was determined by solving the 3 Å structure of a mutant of GBP (AW551) defective in chemotaxis but normal for sugar binding and active transport. This site is in the N-terminal domain and is 18 Å away from the sugar binding site. A novel calcium binding site was discovered during the course of refinement. This site is located in C-terminal domain and resembles the site commonly known as EF-hand, but without the two helices. The site is 30 Å from the sugar binding site and 45 Å from the trg attachment site. The sugar-, calcium-, and membrane-binding sites will be discussed in light of the structure.

02.1-26 THE STRUCTURE OF α-AMYLASE INHIBITOR FROM WHEAT. By Nobuo Tanaka, Youichi Kato, Koji Maeda*, Mamoru Sato, Yukiteru Katsube, Yasuo Hata, Hiroshi Matsubara**, and Tsunehiro Takano*** Inst. for Protein Res., Osaka Univ., Suita, Osaka, Central Res. Lab., Nisshin Flour Co. Ltd., Saitama*, Faculty of Science, Osaka Univ., Toyonaka, Osaka**, and Faculty of Pharmaceutical Science, Setsunan Univ., Hirakata, Osaka***

The structure of the endogenous α-amylase inhibitor from wheat has been studied by an X-ray diffraction method. This inhibitor shows inhibitory activities against α-amylases of various origins. It consists of a single polypeptide chain of 180 amino acid residues and exhibits a high sequence homology with the Kunitz-type trypsin inhibitor of soybean (31%) and winged bean (29%). The prediction by the Chou-Fasmann's method indicated high contents of β-strands (58%).

The inhibitor was crystallized with a microdialysis method. There were two forms of monoclinic crystals in the same vessel. They had the space groups of $P2_1$ with the different unit cell dimensions of a=43.5, b=64.8, c=32.2A, β=113° and a=42.5, b=65.2, c=32.2A , β=112°, respectively. The former is suitable for the structure analysis because it gives the large crystals and sharp diffraction spots beyond 2.0 A resolution. The X-ray intensity data were collected up to 1.8 A resolution for the native crystal and 3.0 A for the two derivatives (K_2PtCl_4 and K_2HgI_4) with anomalous dispersion effects. The current figure of merits was 0.87 with the above data.

The 3.0 A electron density map shows a clear polypeptide folding. The overall structure seems similar to the Kunitz-type protease inhibitor (D.M. Blow et al.(1974) *Biochemistry* 13, 4212-4228) which is rich in β-strands. The analysis of the detailed structure is in progress.