The soluble lytic transglycosylase (SLT) is a monomeric enzyme of 618 amino acids, that is located in the periplasmic space of the *E.coli* bacterium. The enzyme is involved in the metabolism of murein, the structural component of the bacterial cell wall. In vitro, SLT is able to totally degrade intact murein to low-molecular weight muropeptides, much the same as the lysozymes do. However, in addition to cleavage of the β-1,4-glycosidic bonds, SLT carries out a unique transglycosylation reaction forming an internal 1,6-anhydro bond in the muramic acid residue.

Our interest in the structure of SLT has two main reasons. We would like to understand the mechanism by which this enzyme carries out the muramyl transferase reaction and learn more about the role of this enzyme in the metabolism of murein. Moreover, SLT is a potential target for a structure-based design of a new class of antibiotics.

The 3D structure of SLT has been determined by X-ray crystallography to a resolution of 2.7A. The crystal structure was phased to 3.3A using multiple isomorphous replacement. Solvent flattening was used to improve the MIR phases and a preliminary protein model of SLT was constructed from the electron density using the computer graphics software FRODO and O. The protein model was improved and completed stepwise by alternating rounds of partial model refinement with model building in maps calculated from combined MIR, solvent flattened and model phases. The complete model was then refined by simulated annealing using XPLOR. The current crystallographic R-factor is 22.8% for 25115 reflections from 8.0 to 2.7A with $F>2\sigma$.

The protein model of SLT shows a remarkable "doughnut-like" shape. It consists of 3 domains, all very rich in α-helices. The N-terminal domain is formed by 22 interconnecting α-helices in a super-helical arrangement. Via a loop of some 20 residues, this "U-shaped" helical arm is connected to a small linker domain, which packs against the N-terminal end of the protein, thus forming a ring structure with a large central hole of over 20A in diameter. On top of one the sides of this ring, the more globular C-terminal end of the U-domain. Interestingly, this C-terminal domain has a fold which resembles that of the lysozymes, especially that of the goose-type lysozyme. This structural similarity was not expected from sequence analysis. A structural alignment of the C-terminal domain of SLT with different types of lysozymes show that Glu-478 matches the position of the "catalytic" glutamic acid. However, SLT seems to lack the "catalytic" aspartate. The location of the active site of the transglycosylase was confirmed by an inhibitor binding study.

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DS-03.08.09 THE THREE-DIMENSIONAL STRUCTURE OF THE HISTIDINE-CONTAINING PHOSPHOCARRIER PROTEIN HPr FROM *E. coli* AT 2.0 Å RESOLUTION. By Zongchao Jia , E. Bruce Waygood, J. Wilson Quail* and Louis T. J. Delbaere. Departments of Chemistry and Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0

The histidine-containing phosphocarrier protein (HPr) is a central component of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) that transports carbohydrates across the cell membrane of bacteria. The three-dimensional structure of *E. coli* HPr has been redetermined using the method of molecular replacement. The structure has been refined to 2.0 Å resolution with an *R* factor of 0.135 for all data, maintaining very good geometry. The overall folding topology of HPr is a classical open-faced beta-sandwich, consisting of four antiparallel beta-strands and three alpha-helices, and is not compatible with the original *E. coli* HPr X-ray structure (El-Kabbani *et al. J. Biol. Chem.*, 1987, **262**, 12926-12929). As the crystals were obtained from the same condition for the both determinations, the original X-ray structure of *E. coli* HPr is thus likely incorrect. While there is considerable overall structural homology between HPr from *E. coli* and from other organisms, the semi-closed conformation of the active center in this structure is unique but can be correlated to other reported HPr X-ray structures. A comparison between *E. coli* HPr and *Streptococcus faecalis* HPr (Jia *et al. Nature*, 1993, **361**, 94-97) will be presented. In addition, the relationships between some of site-directed mutagenesis results and protein structure/function have been investigated.

PS-03.08.10 REFINED STRUCTURE AND SOLVENT NETWORK OF CHICKEN GIZZARD G-ACTIN DNASE I COMPLEX AT 1.8Å RESOLUTION. By K. SASAKI, K. SAKABE*, N. SAKABE**, H. KONDO***, and M. SHIMOMURA****, College of Medical Technology, Nagoya University, Higashi, Nagoya 461,Japan, *Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan, **National Laboratory for High Energy Physics, Tsukuba, Ibaraki 305, Japan, ***Department of Biology, Faculty of Science, Nagoya Univ. Chikusa, Nagoya 464,Japan,****Faculty of Education,Tokohagakuen University, Sena 1000, Shizuoka 220, Japan.

The refined G-actin structure is essential for the the construction of F-actin and thin filament at atomic level in consideration of atomic informations obtained by other physico-chemical and biochemical techniques and for theoretical experiments.

We refined the crystal structure of chicken gizzard G-actin DNase I complex with 1.8Å resolution data collected on macromolecular data collection system with a Weissenberg camera and imaging plate(IP) at BL6A2 in the Photon Factory (N. Sakabe, Nucl. Instr. and Meth. A303(1991)448-463). The starting model of the coordinates was obtained with five heavy atom derivatives(YB, PCMB, MMA,FMA & BrATP)using MIRA method at 2.5Å resolution. The obtained coordinates were refined using "XPLOR" program. The checking of the atomic positions was carried out using omit maps with PS390 using "FRODO" program. The finding of solvent molecules was carried out on $F_o - F_c$ maps. During the refinement the resolution of the data was increased gradually. The R value is 0.188 including 350 water molecules for 50,000 reflections in 10-1.8Å resolution range.

The average B values of the main chain and side chain atoms for actin are 31.7 and 35.0 respectively. Those of main chain and side chain atoms for DNase I is 14.9 and 17.2 respectively. Thus the motion of actin is 1.5 times larger than that of DNase I. The average B value of main chain atom of DNase I is 11.0 at 2Å resolution reported by Suck et al(J.Mol.Biol.(1986)192,605-632), that is not

