03-Crystallography of Biological Macromolecules

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

so much different from that of DNase I in the complex.
The 350 water positions were assigned on reasonable atomic contact against protein and other water molecules, and about 150 water have small B value than 50. 198 and 139 water molecules have more than one hydrogen bonds, to actin and DNase I, respectively.
Consideration of atomic distance, B value and octahedral coordination, three peaks were assigned as Ca^{2+}ion. One of them is Ca^{2+}binding to ATP of actin and the other two locate in the DNase I region. One of them locates at the same position as Site A in the paper described by Suck.
There are two actin-DNase I contact regions and three actin-actin contact regions. The contact region containing actin 41(G)-44(V) & DNase I 65(T)-67(V) has five solvent bridges between two molecules. Actin-actin contacts are rather weak, the region near to 109(L),112(K),172(H)-176(R) and 370(H)-374(P) forms main contact region and another contact region is between 191(I)-195(R) and 283(K)-286(I) and 374(P). Both regions have two water bridges between adjacent actin molecules.

PS-03.08.11 STUDIES OF MYOSIN LIGHT CHAIN TERTIARY STRUCTURE. By L. Chung*, D.R. Brown#, W. Huang#, B.D. Hambly# & P.M.G. Curmi, *School of Physics, The University of New South Wales, P.O. Box 1, Kensington, N.S.W., 2033, Australia. #Pathology Department, The University of Sydney, N.S.W., 2006, Australia.*

The aim of this project is to produce a model system that facilitates the crystallographic study of the control of myosin force generation by myosin light chains. The regulatory light chain of myosin (LC2) is responsible for the regulation of force generation in smooth- and non-muscle cells. It binds one mole of calcium per mole LC2 , and is activated by phosphorylation. Sequence homology suggests that it is part of the superfamily of calcium binding regulatory proteins that includes calmodulin (CaM) and troponin C (TnC) (Collins,1991 *J. Muscle Res. Cell Motil.* 12: 3-25). Our model system consists of an LC2-synthetic peptide complex that will be suitable for x-ray crystallographic studies. In the long term, we expect to determine the atomic structures of the LC2-peptide complex.
Computer modelling has been used to predict a structural model for the interaction of myosin LC2 with heavy chain (HC). An alpha-helical model of myosin heavy chain was initially built based on a predicted LC2 binding region (Mitchell *et al.*,1989 *J. Mol. Biol.* 208: 199-205). The LC2 portion of the myosin HC sequence was predicted by homology from the binding sequence of myosin light chain kinase given the homology between LC2 and CaM. By means of standard sequence alignment, a sixteen amino acid region of myosin HC for the LC2 binding site was found in the best sequence alignment. This HC segment differs from previous studies. From homology, a model of LC2 was subsequently built by using the x-ray structure of CaM. On the basis of the CaM-peptide complex structure, which has recently been determined (Meador *et al.*,1992 *Science* 257: 1251-5), the model of LC2-HC complex was ultimately made. In this model, the hydrophobic cores of both the N- and C-terminal domains of LC2 naturally interact with the myosin HC hydrophobic residues. HC positive charges fall close to LC2 negative charges. Hence, the predicted binding sequence was supported by modelling the LC2-HC complex.
To verify the results of modelling, biotinylated mutant light chain was mixed with one of a series of 15 residue peptides, that spanned a 50 amino acid region of the myosin heavy chain sequence surrounding the predicted binding site. The peptides differ in sequence by a frame shift of two amino acids. An ELISA-type assay allowed a hierarchy of binding efficiencies to be determined for each of the nineteen 15 residue peptides. From this study, the most likely site for LC2 binding on the myosin heavy chain was determined. We are currently crystallising the LC2-peptide complex.
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03.09 – Receptor and Signal Transducing Proteins

PS-03.09.01 MECHANISM OF THE HYDROLYSIS OF Ras p21.GTP.Mg(H_2O)$_2$.
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The atomic structure of Ras p21 ist stabilized by a great number of intramolecular hydrogen bonds. The structure includes a six stranded beta sheet, five alpha helices and ten loops (H-Ras p21.GppNp.Mg(H_2O)$_{211}$; Symmetry $P3_212$; PDB Dataset 5P21) (E. F. Pai, U. Krengel, G. A. Petsko, R. S. Goody, W. Kabsch, A. Wittinghofer, EMBO J., 1990, 9, 2351-2359). Part of the Ras p21 molecule forms a giant anion hole used as GTP binding site. The triphosphate group of GppNp.Mg(H_2O)$_2$ is held in place by six hydrogen bonds of main chain NH of residues 13 to 18 (loop 1: Gly13-Val14, alpha$_1$ helix: Gly15-Lys16-Ser17-Ala18). Two more hydrogen bonds are formed by Lys16 N, another two by Thr35 (loop 2) and Gly60 (loop 4).
In this crystal structure the conformation of loop 4 is strongly determined by two intermolecular hydrogen bonds between Gln61 (loop 4) to Asp33* and Pro34* (loop 2*) of a neighbor molecule and vice versa because of symmetry. Furthermore the OH group of Tyr32* of this neighbor molecule forms an intermolecular hydrogen bond to O_1 of the gamma phosphate group.
In Ras p21.GDP.Mg.(H_2O)$_{39}$ (Symmetry $P6_522$; PDB Dataset 1Q21) (L. Tong, A. M. de Vos, M. V. Milburn, S.-H. Kim, J. Mol. Biol., 1991, 217, 503-516) loop 2 and loop 4 have different conformations compared to Ras p21.GppNp Mg(H_2O)$_{211}$ and Asp33 as well as Pro34 (loop 2) and Gln61 (loop 4) form different intramolecular hydrogen bonds.
Three water molecules located in the neigborhood of GTP.Mg are necessary for the hydrolysis of GTP.Mg(H_2O)$_2$ to GDP.Mg(H_2O)$_4$ (W175, W189, W171, PDB Dataset 5P21).
The conformational changes of loop 2 and loop 4 caused by the hydrolysis of Ras p21.GTP.Mg(H_2O)$_2$ to Ras p21.GDP.Mg(H_2O)$_4$ have been studied by a time-resolved X-ray crystallographic study of a single crystal (I. Schlichting, S. C. Almo, G. Rapp, K. Wilson, K. Petratos, A. Lentfer, A. Wittinghofer, W. Kabsch, E. F. Pai, G. A. Petsko, R. S. Goody, 1990, Nature, 345, 309-315).
Hydrolysis starts with the movement of the gamma phosphor atom through the plane formed by the three oxygens in the direction of W175 (inversion) (J. Feuerstein, R. S. Goody, M. R. Webb, J. Biol. Chem., 1989, 264, 6188-6190) leading to a transition state where a proton transfer from W175 to O_1 gamma over Gln61 takes place.
A detailed analysis of the observed conformational changes of loop 2 and loop 4 and the conversion of intermolecular hydrogen bonds to intramolecular ones during hydrolysis will be given.
The importance of the highly conserved residues, especially Gly12, for the hydrolysis will also be discussed.
In vivo the rate of hydrolysis is strongly increased by GAP (GTPase activitating protein). We will present a model for possible interaction of GAP by hydrogen bonds with Ras p21 in two different regions of GAP, one "binding domain" and one "docking domain".