dual specificity toward UMP or CMP, while the enzymes of bacteria origin exhibit a more dedicated UMP-specific activity, and appears to be essential for bacterial growth. Thus, bacterial UMPKs may represent potential targets for developing antibacterial drugs. Although several UMPK apo-form structures are available, the ATP-binding and UMP-binding loops are usually flexible and invisible in the apo-form structures. This phenomenon makes it difficult to inspect the induced-fit movements for these flexible loops. Also, no structure has yet been published for the UMPK/GTP complex until to date to get a more thorough understanding of the GTP regulation mechanism. In the present abstract, we have solved the UMPK structures of apo-form and GTP-bound complex form from Xanthomonas campestris using crystals grown under strong magnetic field by X-ray crystallography. We are able to clearly detect the structures of the ATP-binding and UMP-binding loop. Besides, a novel GTP-binding site located in the central hole of the monomers is also detected. Substantial shifting in these two flexible loops is found to be induced when the allosteric effector GTP is bound. Detailed conformational change of UMPK in the presence of allosteric GTP will be discussed.

Keywords: UMPK, allosteric mechanism, GTP regulation mechanism

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Structural studies of novel proteases from the CATH family of zinc peptidases

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Proteins in the CATH family of zinc peptidases (phosphorylase/hydrolase-like fold in SCOP) have a broad phylogenetic spread across all kingdoms of life and show substantial sequence divergence. They are in 8 PFAM families and form the large peptidase_MH clan. Despite several structures in the PDB, only half of the members have reliable homology models. The JCSG aims to improve this coverage by determining novel structures. HMMs were used to identify 226 members with cDNA available in the JCSG genome pool. Of these, 161 have <30% sequence homology to a structure in PDB. After clustering at 90% sequence identity to remove close homologs, 135 targets were chosen. To date, 8 targets have been solved, with 6 others in crystallization trials. We have analyzed features that support different functions, focusing on active sites, ligands, domain architectures and oligomerization. Even with a modest increase in structural coverage, we could assign new functional roles within the clan and more clearly discern the evolutionary connections in its PFAM families. We also identified many proteins of biomedical importance. Four structures can be used to model ~130 proteins in prevalent pathogenic bacteria and may allow the design of new therapies. Two carboxypeptidases are close homologs of an enzyme that is used in prodrug and cancer therapy. An AstE/AspA-like member is related to a protein involved in a brain disease. We also obtained the first structure of an aminopeptidase with irons bound in the active site, which hints at functional novelty. A putative Xaa-His dipeptidase represents the first structure of a PepD and reveals a dimeric form. The JCSG is funded by NIGMS/PSI, U54 GM074898. SSRL is funded by DOE BES, and the SSRL SMB program by DOE BER, NIH NCRR BTP and NIH NIGMS.

Keywords: structural genomics, structural biochemistry enzymology, zinc peptidase

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Structural and functional analysis of a universal stress protein from *Thermus thermophilus* HB8

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The universal stress protein (Usp) superfamily [Pfam PF00582] is characterized by a conserved domain consisting of 130-160 amino acids. More than 1000 Usp proteins are found in various organisms including bacteria, archaea, and eukaryotes. Escherichia coli possesses six proteins containing a Usp domain. They are induced under a large number of stress conditions; nutrient starvation, heat shock, oxidants, uncouplers, and DNA-damaging agents. However, the biochemical mechanism of Usp proteins remains unknown. The genome sequence of the extremely thermophilic bacterium Thermus thermophilus HB8 has revealed that five proteins belong to the Usp superfamily. Two are in a single domain, two are in tandem, and one is a component of the tentative potassium uptake protein TrkA. TTHA0895 is a single domain Usp protein from Thermus thermophilus HB8 and consists of 137 amino acid residues with a molecular mass of 14759 Da. In order to determine its structural properties, TTHA0895 was crystallized in the absence and presence of ATP. Form I, crystallized in the absence of ATP, belongs to tetragonal space group $P4_32_12$ with unit-cell parameters $a = b = 73.1$, $c = 57.9$ Å, and form II, crystallized in the presence of ATP, belongs to orthorhombic space group $I222$ with unit-cell parameters $a = 33.1$, $b = 75.1$, $c = 88.7$ Å. The crystals contain one monomer per asymmetric unit. X-ray data have been collected to 1.65 and 1.55 Å resolution for forms I and II, respectively. Here we report the X-ray structures of forms I and II, and the possible ATPase activity of TTHA0895. In addition, the expression of TTHA0895 from the log phase to the stationary phase of bacterial growth has been examined by means of mRNA (microarray) analysis. The presence of tetracycline had no effect on TTHA0895 regulation.

Keywords: structure and function of proteins, structures of biomolecules, structural genomics

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Crystal structure and molecular dynamics simulation of ubiquitin-like domain of murine Parkin

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Parkin is the gene product identified as the major cause of autosomal

recessive juvenile parkinsonism (AR-JP). Parkin contains a unique ubiquitin-like domain in its N-terminus designated Uld which is assumed to be a interaction domain with the Rpn 10 subunit of 26S proteasome. To elucidate the structural and functional role of Uld in parkin at the atomic level, the X-ray crystal structure of murine Uld was determined and a molecular dynamics simulation of wild Uld and its five mutants (K27N, R33Q, R42P, K48A and V56E) identified from AR-JP patients were performed. Crystals of Uld were obtained by the hanging-drop vapor-diffusion method using NaCl as a precipitant. Diffraction data were collected to 1.65Å resolution. The structure of Uld was determined by the single-wavelength anomalous diffusion (SAD) method using an iodinated derivative. The final model gave the R-factor of 0.195 and R_{free}-factor of 0.244. Murine Uld consists of two α helices and five β strands, and its overall structure is essentially the same as that of human ubiquitin with a 1.22 Å rmsd for the backbone atoms. The MD simulations showed the K27N and R33Q mutations increase the structural fluctuation of these β strands including the α1 helix. Reversely, the V56E mutant restricted the spatial flexibility at the periphery of the short α2 helix by the interactions between the polar atoms of Glu56 and Ser19 residues.

Keywords: Parkin, ubiquitin-like domain, molecular dynamics simulation

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Structural and functional whole-cell project for the model organism, *Thermus thermophilus* HB8

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This research project aims to understand all fundamental biological phenomena at an atomic-resolution, on the basis of molecular structures and functions. Towards this aim, we selected the extremely thermophilic organism, *Thermus thermophilus* HB8, as a model organism, because many of the approximately 2,200 genes encoded in its genome have been selected during evolution and are common to many organisms. However, about 500 of the genes (proteins) are functionally-uncharacterized. As a first step to obtain functional clues about these proteins, we determined their three-dimensional structures. Based on the structures, we inferred the molecular functions of about 60% of them and intensively characterized several family proteins, such as the house-cleaning NUDIX hydrolases, metallo-beta-lactamases and DNA repair proteins. While we have continued to solve the structures of other uncharacterized proteins for their functional inference, we have also been exploring their functions by functional genomics analyses (mRNA, protein and metabolite) in combination with gene disruption and stress-perturbation. For example, we found that cyclic AMP receptor protein (CRP), which is known as a global transcriptional factor, regulates 22 genes, including ones presumably involved in host defense (1 characterized and 21 uncharacterized), whereas one of the CRP family proteins functions in stationary phase, and regulates 14 genes related to energy and redox metabolism (3 characterized and 11 uncharacterized). We also found that about 40 genes of unknown function display altered mRNA expression upon metal stress. All of the plasmids for protein expression and gene disruption prepared in our laboratory are now available from the RIKEN BioResource Center (see http://www.thermus.org/).

Keywords: structural genomics, functional genomics, systems biology

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X-ray crystal structure of a hypothetical Sua5 protein from *Sulfolobus tokodaii* strain 7

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The Sua5-yciO-yrdC domain proteins are widely distributed in prokaryotes and eukaryotes. One of the proteins in this family, *Escherichia coli* YrdC, preferentially binds to double-stranded RNA and DNA. It has been predicted to be a rRNA maturation factor. Sua5 consists of an N-terminal YrdC domain and a C-terminal Sua5 domain. The *sua5* gene was first identified in *Saccharomyces cerevisiae* as a suppressor of a translation initiation defect of the iso-1-cytochrome c (*cyc1*) gene. The function and 3D structure of Sua5 remain to be elucidated. In the present study, we determined the crystal structure of Sua5 (ST1526) from thermoacidophilic archaeon *Sulfolobus tokodaii* strain 7, which exhibits 49.7% similarity to *S. cerevisiae* Sua5. The overall fold of the N-terminal yrdC domain of *Sulfolobus* Sua5 is similar to that of *E. coli* YrdC, the *Z*-score being 21.3 and the r.m.s.d. value being 2.4 Å. A large concave surface exhibiting a positive electrostatic potential, which is similar to that in YrdC, was found in Sua5. Interestingly, excess electron density that might be due to an *E. coli*-derived nucleotide was observed on this concave surface. The C-terminal Sua5 domain consists of three α-helices and five β-strands, which adopt a Rossmann fold. A structure similarity database search using the DALI server revealed that the closest structure was that of *Methanocaldococcus jannascii* HypB, a GTP-binding protein that regulates metal binding. Thus, the three-dimensional structure of Sua5 showed that both the N- and C-terminal domains might be involved in nucleotide binding or metabolism, which is supported by the observation that Sua5 showed ATP hydrolysis activity, AMP being produced.

Keywords: nucleotide, Rossmann fold, translation factor

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Characterization of metal ions and protein oligomeric states in JCSG structures

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