is cleaved for up to 75 residues during crystallization automatically. The final core structure reveals a novel hybrid comprising a tudor-like (Sm-like) domain interdigitated with a Immunoglbolubin-like (Ig) or fibronectin type (Fn) III domain. In the crystal, the monomers form an annular pentamer of dimers of pseudo five-fold symmetry, due to different dimer-dimer interactions employed. The resulting asymmetrical star-like complex has a outer dimensions of approximately 110 Å × 90 Å × 65 Å, and a shortest diameter of approximately 20 Å; in the center. The outer dimensions of the atomic Xanthomonas hook-capping FlgD complex turn out to be very similar to those of the Salmonella filament cap complex observed by electron microscopy. This atomic hook cap structure may help understand hook protein-cap protein interactions, hook protein insertion, and hook length control, the three features that are crucial for understanding the bacterial flagellar biogenesis.

Keywords: FlgD, hook capping, flagellar biogenesis

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Structure of DFA0005 complexed with α-ketoglutarate: A novel member of the ICL/PEPM superfamily

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The pale-pink alkali-tolerant Deinococcus ficus (DF strain CC-FR2-10T) is a novel bacterium isolated from the rhizosphere of the sacred tree Ficus religiosa L. It is found to possess high degrading power for food and organic waste at pH 10 and is being applied to industrial usage. It contains one chromosome of 2801970 bp, and three mega-plasmids of 642681, 395118, 311736 bp, respectively. The total genome sequence has been recently completed (Lin et al, manuscript in preparation), and a structural genomics project is followed to study the structures of proteins encoded in this genome and to study the alkali adaptation mechanism exhibited by this alkali-tolerant bacterium compared with their non-alkaline counterparts. The crystal structure of the DFA0005 protein complexed with α-ketoglutarate (AKG) from an alkali-tolerant bacterium Deinococcus ficus has been determined to a resolution of 1.62 Å. The monomer forms an incomplete α7/ β8 barrel, with a protruding α8 helix that interacts extensively with another subunit to form a stable dimer of two complete α8/ β8 barrels. The dimer is further stabilized by four glycerol molecules situated at the interface. One unique AKG ligand binding pocket per subunit is detected. Fold match using the DALI and SSE servers identifies DFA0005 as belonging to the isocitrate lyase / phosphoenolpyruvate mutase (ICL/PEPM) superfamily. However, further detailed structural and sequence comparison with other members in this superfamily and with other families containing AKG ligand indicate that DFA0005 protein exhibits considerable distinguishing features of its own and can be considered a novel member in this ICL/PEPM superfamily.

Keywords: Deinococcus ficus, ICL/PEPM superfamily, alpha-ketoglutarate ligand

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RecX adopts a tandem repeats of three-helix bundle:Insights into RecX inhibition of RecA activities

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The bacterial RecA protein plays a key role in recombinational DNA repair in bacteria. it is a DNA-dependent ATPase that promotes DNA strand exchange reactions, and induces SOS response. However, the activities of RecA protein must be regulated in the cell to avoid aberrant DNA recombination. Currently, many additional proteins are known to regulate RecA function such as RecF, RecO, and RecR and DinI proteins. RecX protein is an inhibitor of RecA function both in vivo and in vitro. However, RecX protein is among the least understood of these RecA modulator proteins. But it has been found that RecX protein inhibits RecA protein ATPase, coprotease, and recombinase activity. Direct interaction between RecA and RecX has also been well demonstrated. Reconstituted RecA/RecX nucleoprotein filament from EM and RecA crystal structure show that RecX protein bind to the helical groove of RecA filament, spanning the monomer-monomer interface from the C-terminal domain of one RecA monomer to the core domain of the second. However, detailed RecX crystal structure is still unavailable until to date. We have solved the first RecX crystal structure from Xanthomonas campestris to a resolution of 1.6 Å; crystallography. The tertiary structure of RecX indicates it adopts a novel three tandem repeats of three helix bundle. Enzyme assays indicate that RecX can efficiently inhibit the ATPase, coprotease, and recombinase activity of the RecA protein. A docking study of RecA dimer and RecX monomer reveals the tight interactions between these two proteins. Model study shows that RecX proteins can fit well into the major helical groove, with its C-terminus fitted into the notch between two adjacent RecA monomers in the RecA nucleofilament, as revealed by the cryoEM study.

Keywords: RecA, RecX, recombination

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Structure of Xcc UMPK/GTP complex reveals a novel GTP-binding site and allosteric mechansim

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Uridylate kinase (UMPK) is a key enzyme in pyrimidine biosynthesis. Due to its importance in nucleic acid biosynthesis, UMPK is ubiquitous in every living organism, including bacteria, archaea, and eukarya. However, UMPKs of bacterial origin are very different from the UMPKs of eukaryotic origin. In general, the eukaryotic enzymes have a broader substrate preference, exhibiting

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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