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Structural basis for Ufm1 processing by UfSP1

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Ubiquitin fold modifier 1 (Ufm1) is a newly identified ubiquitin-like protein. Like ubiquitin and other ubiquitin-like proteins, Ufm1 is synthesized as a precursor that needs to be processed to expose the conserved C-terminal glycine prior to its conjugation to target proteins. Two novel proteases, named UfSP1 and UfSP2, have been shown to be responsible for the release of Ufm1 from Ufm1-conjugated cellular proteins as well as for the processing of its precursor. They show no sequence homology with known proteases. Here, we describe the 1.7Å resolution crystal structure of mouse UfSP1, consisting of 217 amino acids. The structure reveals that it is a novel cysteine protease having a papain-like fold, with Cys53, Asp175, and His177 that form a catalytic triad, and Tyr41 that participates in the formation of the oxyanion hole. This differs from the canonical catalytic triad of papain-like proteases in that the aspartate and the histidine residues are from the 'Asp-Pro-His' box. The Asp-Pro-His configuration seen in UfSP1,together with Atg4B and M48USP, seem to form a new subfamily of the cysteine protease superfamily. The mutagenesis study of the active site residues confirms structural basis for catalysis. The interaction between UfSP1 and Ufm1 appears quite substantial, since the KD value was estimated to be 1.6 uM by the isothermal titration calorimetry analysis. Furthermore, theNMRdata shows that the loop between b3 and a2 in addition to the C-terminal region of Ufm1 plays a role in binding to UfSP1.

Keywords: Ufm1, UfSP1, DUB

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Crystal structure of the muramidase domain of FlgJ, a putative flagellar rod cap protein

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The axial structure of the bacterial flagellum consists of three parts: the filament as a helical propeller; the hook as a universal joint; and the rod a drive shaft connecting the hook and the MS ring. The construction of the axial structure, which occurs at its distal end, requires cap complexes attached to the growing end. FlgD and FliD are cap proteins necessary for hook and filament growth, respectively. Because the rod penetrates the peptidoglycan (PG) layer, the rod cap is thought to have a PG-hydrolyzing activity. FlgJ is a putative rod cap protein. The N-terminal region of FlgJ interacts with the rod proteins and the C-terminal region shows a sequence similarity to muramidase, such as autolysin, muramidase2 and AcmA. To understand the mechanisms of rod formation, we solved the crystal structure of a C-terminal fragment of FlgJ at 1.7 Å; resolution using diffraction data collected at SPring-8 beamline BL41XU. The putative active site structure is similar to that of lysozyme, although no significant sequence similarity is found between FlgJ and lysozyme. Based on the structure, we identified residues essential for the PG-hydrolyzing activity and confirmed them by mutational analyses.

Keywords: bacterial flagellum, muramidase, crystal structure

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The three dimensional structure of an intact glucoamylase

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The three dimensional structure of a complete Hypocrea jecorina glucoamylase has been determined at 1.8 Å resolution. Previous structures of other fungal and yeast glucoamylase catalytic and starch binding domains have been determined separately, this is the first intact structure that allows visualization of the juxtaposition of the starch binding domain relative to the catalytic domain, including the 37 residue linker segment. The detailed interactions we see between the catalytic and the starch binding domains are confirmed in a second independent structure determination of the enzyme in a second crystal form. This second structure model exhibits an identical conformation compared to the first structure model. The proposed starch binding regions for the starch binding domain are aligned with the catalytic domain in the three-dimensional structure in a manner that supports the hypothesis that the starch binding domain serves to target the glucoamylase at sites where the starch granular matrix is disrupted and where the enzyme might most effectively function.

Keywords: glucoamylase, carbohydrate binding module, Hypocrea jecorina

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Allostery and functional refolding in the Gram-negative hexameric Type II citrate synthases

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In most organisms, citrate synthase (CS) is a dimer of identical subunits (approx. 400 amino acids). This enzyme catalyzes the entry point of carbon (in the form of acetyl-CoA) into the tricarboxylic acid cycle, a critical energy-producing metabolic process. This form of CS is designated Type I and is not regulated. In contrast, Gram-negative bacteria have a different kind of CS, designated Type II, which form hexamers that are allosterically inhibited by NADH. Our structural studies of the Gram-negative E. coli Type II CS (426 amino acids) identifies a reversible folding element (residues 262-298) adjacent to the active site that controls functionality, and which is unfolded

in the T-state (inactive; NADH bound). Notably, in the unregulated Type I CS these same residues form a stable folded region composed of two large helices that are a key part of the binding site for acetyl-CoA. Kinetic analyses of both types of CS indicate a similar catalytic mechanism, suggesting that in the active R-state of E. coli Type II CS, the conformation of residues 262-298 will be similar to that seen in the unregulated Type I enzymes. This uniquely evolved functionality switch in the Type II CS provides the opportunity to study a novel aspect of the protein folding problem, namely how to engineer the property of reversible instability into a formerly stably folded region. We have specifically designed and determined the structures of variant enzymes to gain a better understanding of the mechanistic features of this allosterically controlled regulatory folding/unfolding system. This has resulted in a series of stepwise structural snap shots of the refolding process in going from the T to R-states in Type II CS. Supported by the Natural Sciences and Engineering Research Council of Canada.

Keywords: enzyme allostery, protein folding, enzyme regulation

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Crystal structure analysis of human membrane integrated protein leukotriene C_4 synthase

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Human membrane integrated protein is one of the most exciting target for protein crystallographers, due to the difficulties in protein preparation as well as crystallographic work. In the year 2007, the structures of three human membrane proteins were determined crystallographically first. Leukotriene C_4 synthase (LTC_4S) is one of the three human membrane integrated proteins. LTC_4S, which is a membrane integrated protein existing in nuclear membrane, catalyzes the conjugation between leukotriene (LT) A_4 and glutathione (GSH) to form LTC_4. LTC_4 and its metabolites LTD_4 and LTE_4 are components of slow reacting substance of anaphylaxis, and they are called cysteinyl leukotrienes (Cys-LTs) because they have a cysteine moiety commonly. Cys-LTs are lipid mediators involved in smooth muscle constriction and inflammation, particularly in asthma. LTC_4S is the membrane protein responsible for the biosynthesis of Cys-LTs and a potential target for drug discovery. The crystal structure of human LTC_4S was determined at 3.3Å resolution using the recombinant LTC_4S. We established the over-expression system using *Schizosaccharomyces pombe*, and we also used the expression system for the preparation of the selenomethionine derivative of LTC_4S with a Leu121Met mutation for the MAD phase calculation as well as the native LTC_4S with a histidine tag. In the crystal structure LTC_4S forms trimer structure, and there is a V-shape substrate binding cleft between two adjacent monomers. GSH, which is one of the substrates, was co-crystallized with LTC_4S, bound at the upper space of the V-shape cleft. Based on the crystal structure, we proposed the acid-base catalytic mechanism, and Arg31 from a monomer and Arg104 from the other monomer exert the acid and base, respectively.

Keywords: membrane protein structures, X-ray crystallography of biological macromolecules, drug targets

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Structural insights into substrate specificity of isomaltase from *Saccharomyces cerevisiae*

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Oligo-1,6-glucosidase (dextrin 6-α-D-glucanohydrolase; EC 3.2.1.10) generally hydrolyzes nonreducing terminal α-1,6-glucosidic bonds of isomaltooligosaccharides and α-limit dextrin more rapidly than those of isomaltose. However, oligo-1,6-glucosidase from *Saccharomyces cerevisiae* (isomaltase) preferentially hydrolyzes isomaltose and weakly acts on isomaltooligosaccharides. To understand the difference in substrate specificity between isomaltase and other oligo-1,6-glucosidase from a structural view point, the crystal structure of isomaltase and isomaltase complexed with maltose as a competitive inhibitor were determined. Isomaltase was crystallized by the hanging drop vapor diffusion method using PEG3350 as a precipitant. An inhibitor complex was cocrystallized under the same conditions in the presence of 0.2 M maltose. Data sets for structural analysis were collected on synchrotron radiation to 1.6 Å resolution for the native and maltose complex. The crystals belong to the monoclinic space group *C*2 with the cell dimensions *a*=95.7 Å, *b*=115.4 Å, *c*=61.8 Å, β=91.2° and one 67 kDa molecule per asymmetric unit. The Overall structural features of isomaltase are similar to those of other GH family 13 enzymes such as α-amylases and *Bacillus cereus* oligo-1,6-glucosidase. It consists of three domains containing a $(\beta/\alpha)_8$ barrel structure. This reveals that the entrance of the active site cleft is narrowed by Tyr158, His280, and a loop between 310 to 315, thus disaccharide binds most efficiently in the active site for hydrolysis. The glucose residue of the nonreducing end is buried deeply at the bottom of the cleft, and tightly bound by nine hydrogen bonds and a stacking interaction with Tyr72. However, no structural change is observed before and after maltose binding.

Keywords: glycosyl hydrolases, isomaltase, substrate specificity

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Mechanism for formation of Arg-AMP in help of tRNA on the basis of structure of ArgRS, tRNA and ATP

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In arginyl- and glutaminyl-tRNA synthetases (ArgRS, GlnRS) from investigated all species, no ATP-PPi exchange reaction is observed in the absence of cognate tRNA and in the presence of their tRNA treated with periodate. The pyrophosphorolysis reaction of synthetic Arg-AMP is also not observed in the absence of tRNA. The detailed mechanism through which 2'OH of the ribose of the 3' end Ade76 of the cognate tRNA accelerates these reactions has been remained