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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 1.7 Å 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 μM by the isothermal titration calorimetry analysis. Furthermore, the NMR data 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 formation and filament assembly, 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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**Allosteric and functional re-modelling 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.