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Structural and biochemical characterization of ClpP from *Bacillus subtilis*

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The ATP-dependent chaperone/protease complex, ClpAP or ClpXP, plays an important role in the protein degradation in most bacteria or in mitochondria and chloroplast of eukaryotes. ClpP is a proteolytic component that has 14 identical subunits organized in two stacked heptameric rings whereas ClpA and ClpX is a hexameric AAA+ ATPase that binds, denatures, and trasnlocates protein substrates. We have solved the crystal structure of ClpP from *Bacillus subtilis* (BsClpP) at 2.4 Å resolution and tested proteolytic activity against peptide and protein substrates. The structure of BsClpP was determined by molecular replacement method using that of *E. coli* ClpP (EcClpP), which is highly characterized ClpP and has 68% homology to BsClpP. The model of BsClpP has been refined to crystallographic *R* and *R-free* factors of 24.4 and 28.8 % and shows very similar overall structure with EcClpP. Although the structural and sequential resemblance between *E. coli* and *B. subtilis* species is significantly high, ClpX from *E. coli* is not able to stimulate the proteolytic activity of BsClpP and ClpX from *B. subtilis* also is not able to stimulate that of EcClpP. It is in stark different to a similar bacterial ATP-dependent protease, HslVU which shows mutual activation through swapped combination.

Keywords: ClpP, proteolytic enzymes, *Bacillus subtilis*

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Implications for selenophosphate generation by crystal structure of selenophosphate synthetase

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Selenium is an essential micronutrient incorporated into proteins and RNAs, as selenocysteine (Sec) and 2-selenouridine residues, respectively. For both of the Sec synthesis and RNA modification reactions, selenophosphate is utilized as a reactive selenium donor. Selenophosphate synthetase (SPS) is the enzyme responsible for the generation of selenophosphate, by activating selenide with ATP. Many SPSs are themselves Sec-containing proteins, in which Sec replaces the catalytically essential Cys residue (designated Sec(Cys)). We solved the crystal structures of a bacterial SPS, and performed mutational analyses. The structure of SPS complexed with α, β-methylene ATP (AMPCPP) revealed the ATP-binding site, formed at the subunit interface of the homodimer. Four Asp residues coordinate four metal ions to bind the AMPCPP phosphate groups. The conformations of the N-terminal loop, including the essential Sec(Cys) and Lys residues, differ between the two subunits (open and closed). The AMPCPP γ-phosphate group is solvent-accessible, and a putative nucleophile could attack it to generate selenophosphate and ADP. Selenide attached to the Sec(Cys) residue, as a perselenide(selenosulfide) group, could be the nucleophile, upon closing the N-terminal loop. A well-ordered water molecule, fixed close to the β-phosphate group, could be the nucleophile in the subsequent ADP hydrolysis to orthophosphate and AMP.

Keywords: SelD, selenoprotein, PurM superfamily

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The crystallographic study of the deubiquitinating enzyme UCH37 N-terminal domain

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Ubiquitin is a highly conserved protein in eukaryotes and it is used as a label of the target protein for a proteolysis with 26S proteasome in ubiquitin-proteasome system. After degradation, the ubiquitin chain is removed from target protein by deubiquitinating enzyme and recycled. The deubiquitinating enzymes are cystein proteases. They are classified into two groups: ubiquitin carboxyl-terminal hydrolases and ubiquitin-specific proteases. UCH37 belongs to the ubiquitin C-terminal hydrolases family. The N-terminal domain of UCH37 (1-237) is a catalytic domain, the C-terminal domain (238-329) is able to interact to hRPn13 of 26S base subunit via KEKE motif (312-329). The C-terminal domain is not conserved in ubiquitin carboxyl-terminal hydrolase family and would be expected to form a unique conformation to UCH37. To elucidate the mechanism of deubiquitin activity and interaction with 26S by based on the structure, we have tried to determine the structure of UCH37 using X-ray crystallography. At first, the UCH37 could be expressed as a GST fusion protein and purified using affinity chromatographic method. However, the purified sample was digested to N- and C-terminal domains with time. Therefore, we had tried to express the N- and C-terminal domains separately. The N-terminal domain was able to construct a useful expression and purification system, and then the crystal had been obtained using a polyethylene glycol as a precipitant. We had harvested the native dataset over the 2.9Å resolution in SPring-8. We will report these expression, crystallization and crystallographic data. Now, we are trying to improve the crystal quality and to determine the phase using molecular replacement method. We will also discuss these results in this conference.

Keywords: ubiquitin system, proteases, proteasome

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Flexibility in active site of *cis*-3-chloroacrylic acid dehalogenase revealed by multiple structures

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