

the Walker B motif form a novel  $3_{10}$  helix, which is not observed in other ABC ATPases. This results in an unorthodox conformation of a conserved glutamate residue involved in ATP hydrolysis. Compared to other ABC-ATPase structures, significant displacement occurs at a linker region between the ABC  $\alpha\beta$  and  $\alpha$  helical domains, leading to an atypical surface structure around the Q loop. This surface feature suggests that SufC interacts with SufB and SufD in a different manner from that observed in the structure of ABC transporters.

**Keywords:** ABC-ATPase, structural biology, Suf protein

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#### Comparisons of the Structures of Isolated Proteolytic Domains of Lon Proteases

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Crystal structure of the proteolytic domain of *A. fulgidus* B-type Lon protease (*AfLonB*) revealed significant differences in the conformation of the active site compared to two other known Lon P-domains, from *E. coli* (*EcLonA*) and *M. jannaschii* (*MjLonB*) [1], despite the similarity of the overall fold. The differences in the interactions of the catalytic residues in the active sites of *AfLonB* and the other two Lon proteases are primarily connected to the variable conformational state of the segment that precedes catalytic Ser509. It appears that in isolated P-domains of single chain Lon proteases this segment does not have a stable conformation that could maintain proper structure of the active site. Other ATP-dependent proteases with known structures, such as HslUV or ClpAP, are two-chain enzymes, and in their independently-folded proteolytic subunits the catalytic residues are in appropriate positions. We suggest that the interactions with other domains (the ATPase domain in particular), as well as ligand binding, might lead to rearrangements in Lon P-domain active sites. Full-length *AfLonB* is proteolytically active in an ATP-dependent manner, whereas all individually purified wild-type and mutant P-domains are inactive. These results suggest that the structure of the active site in the P-domain of *AfLonB* represents an inactive state of enzyme. This raises the possibility that the surprising differences between the catalytic mechanisms of A and B type Lon proteases [1] might be artifacts, since the structure of the P-domain of *MjLonB* could similarly represent an inactive state of that enzyme.

[1] ImY. J., et al., *J. Biol. Chem.*, 2004, **279**, 53451.

**Keywords:** Lon proteases, active site, structure comparison

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#### Structures and Function Studies of Microbial P-loop-containing Phosphatases

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The P-loop-containing phosphatases are composed of a conserved sequence of DX(30)HCXXGXXR(T/S). It is well-known that tyrosine phosphatases (PTP), dual specificity phosphatases (DSP) and inositol polyphosphate (IPP) phosphatase are important regulators in signal transduction of cell cycle and IPP signaling molecules. Up to date, we have solved two the P-loop-containing phosphatases which are the inositol hexakisphosphate phosphatase (phytase) from *Selenomonas ruminantium* and DSP in *Sulfolobus Solfataricus*. We have solved two crystal forms of the complex structure of the phytase with an inhibitor, *myo*-inositol hexasulfate. In the "standby" and the "inhibited" crystal forms, the inhibitor is bound, respectively, in a pocket slightly away from Cys241 and at the substrate-binding site where the to-be-hydrolyzed phosphate group is held close to the -SH group of Cys241.

P-loop-containing phosphatase from *S. Solfataricus* was also solved. Comparison of the structures of *S. Solfataricus* and other

phosphatases have revealed a extensive substrate binding surface which implying the possibility of low specificity. Overall, these investigations help us to evaluate the evolution of tyrosine phosphatase in microbial and the role it plays in the signal transduction among Achaea.

**Keywords:** dual specificity phosphatase, phytase, *myo*-inositol polyphosphates

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#### Structure of a Two-domain Chitinase from *Streptomyces griseus*

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Many glycoside hydrolases consist of multiple domains involved in catalysis and carbohydrate binding, which are connected by interdomain linkers. The widespread occurrence of these linkers suggests their importance in the binding and catalytic functions. However, the number of available structures of full-length glycoside hydrolases has been limited. We first revealed the whole structure of a two-domain chitinase, namely chitinase C from *Streptomyces griseus* HUT6037 (ChiC), classified into glycoside hydrolase family 19. ChiC is composed of an N-terminal chitin-binding domain, a C-terminal catalytic domain, and a linker peptide. Although the cubic crystals of full-length ChiC contain two molecules in an asymmetric unit, the electron densities are assigned to three out of the four domains because of incomplete densities. While the electron densities for the two catalytic domains are clearly defined, that corresponding to the single chitin-binding domain is obscured. This absence and obscurity of electron densities is presumably caused by conformational flexibilities of the linker peptides. The two discrete domains, chitin-binding and catalytic domains, connected by the linker peptide are distantly located without any interactions with each other in the crystal. Great flexibility of the linker must allow the two separated domains to be close to each other in solution, and hence cooperation between the domains is likely to be important for the full activity.

**Keywords:** chitinase, whole structure, conformational flexibility

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#### Structure-based Functional Analysis of Prenyltransferases: *Trans*-type OPPs from *T. maritima* and *Cis*-type UPPs from *E. Coli*

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Isoprenoids are an extensive group of natural products with diverse structures consisting of various numbers of five-carbon isopentenyl pyrophosphate (IPP) units. The enzymes responsible for the synthesis of linear isoprenyl pyrophosphates can be classified as *cis*- and *trans*-isoprenyl pyrophosphate synthase (IPPs) according to the stereochemical outcome of their products. The C<sub>40</sub> product of octaprenyl pyrophosphate synthase (OPPs) constitutes the side chain of ubiquinone in *Thermotoga maritima*. Among the *cis*-polyprenyl pyrophosphates, the C<sub>55</sub> product of the bacterial undecaprenyl pyrophosphate synthase (UPPs) serves as a lipid carrier in cell wall peptidoglycan biosynthesis.

OPPs is composed entirely of  $\alpha$ -helices joined by connecting loops and is arranged with 9 core helices around a large central cavity. An elongated hydrophobic tunnel between D and F  $\alpha$ -helices contains two DDxD motifs on the top for substrate binding and is occupied at the bottom with one large residue F132. From the biochemical studies, F132 is the key residue for determining the product chain length.

The structures of *Escherichia coli* UPPs were determined previously as an apo-enzyme, in complex with Mg<sup>2+</sup>/sulfate/Triton, and with bound FPP. In further search of its catalytic mechanism, the