large cavity is formed at the dimer interface and extends between the two domains of each subunit. Binding studies have demonstrated the ability of this protein construct to bind to cellulose.

These observations suggest a broadening in the functional repertoire of CcdA-like proteins and possible role in carbohydrate processing.

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Keywords: protein structures, structural biology, carbohydrate binding proteins

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Structure of the N-terminal domain of PEX1 AAA-ATPase

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Peroxisomes are responsible for several pathways in primary metabolism, including beta-oxidation and lipid biosynthesis. PEX1 and PEX6 are hexameric AAA-type ATPases, both of which are indispensable in targeting over 50 peroxisomal resident proteins from the cytosol to the peroxisomes. Although the tandem AAA-ATPase domains in the central region of PEX1 and PEX6 are highly similar, the N-terminal sequences are unique. To better understand the distinct molecular function of these two proteins, we analyzed the unique N-terminal domain (NTD) of PEX1. Extensive computational analysis revealed weak similarity of PEX1 NTD to the N-terminal domains of other membrane related type II AAA-ATPases, such as VCP / p97 and NSF. We have determined the crystal structure of mouse PEX1 NTD at 2.05 Å resolution, which clearly demonstrated that the domain belongs to the double-psi-barrel fold family found in the other AAA-ATPases. The N-domains of both VCP and NSF are structural neighbors of PEX1 NTD with a 2.7 Å and 2.1 Å r.m.s.d. of backbone atoms, respectively. Our finding suggest that the supra-domain architecture, which is composed of a single N-terminal domain followed by tandem AAA domains, is a common feature of organellar membrane-associating AAA-ATPases. We propose that PEX1 functions as a protein unfoldase in peroxisomal biogenesis, using its N-terminal putative adaptor-binding domain.

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Hyperthermostable Ferredoxin from *Pyrococcus furiosus*

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Pyrococcus furiosus is a hyperthemophilic archaeon with a growth optimum of 373 K. Its ferredoxin is a 66 amino acid electron transfer protein, which contains one $[Fe_4S_4]$-cluster. An aspartate residue is coordinating one of the irons, which is easily lost so that the cluster converts to an $[Fe_3S_4]$-cluster under oxidizing conditions. The protein also contains a disulfide bond, which is redox active at approximately the same potential as the $[Fe_4S_4]$-cluster and exists in equilibrium between two conformations [1].

The structure of *pyrococcus furiosus* ferredoxin was determined to 1.5 Å by molecular replacement with ferredoxin from *Termotoga maritima* [2], [3]. It reveals an extensive hydrogen-bonding network, which provides an explanation for the thermostability. It has been suggested that *pyrococcus furiosus* ferredoxin is a dimer under physiological conditions [4]. The packing of the two molecules in the asymmetric unit indicates the intermolecular contacts in such a dimer. The disulfide bond is seen in two conformations.

Recently, we have managed to crystallize the $[Fe_4S_4]$-form of the protein. We are currently working on optimizing these crystals.

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Keywords: metalloprotein, iron sulfur cluster, thermostable proteins

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Crystal Structure of the NgcE Protein of the *Streptomyces* ABC transporter

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The NgcE protein binds *N*-acetylglucosamine (GlcNAc) as well as *N,N'*-diacetylchitobiose and is a component of the ABC transporter Ngc for GlcNAc-uptake in *Streptomyces olivaceoviridis*. The NgcE protein was overproduced in a soluble and purified to homogeneity. Crystals of NgcE, which grew in the presence of 1 m*M* GlcNAc, 20 %(w/v) PEG MME 2000, and 100 mM Tris-HCl (pH 8.5), showed plate-like form, and belonged to either space group $P2_12_12$ (a=59.9, b=153.0, c=41.7 Å) or $P2_12_12_1$ (a=58.1, b=96.3, c=151.7 Å). The former crystals diffracted to 2.2 Å resolution and the latter to 1.8Å. The MAD phasing and the initial model building were performed using 2.0Å data sets of a selenomethionine-derivative $P2_12_12_1$ crystal. The structure of the NgcE protein containing GlcNAc was solved as well as the structure containing *N,N'*-diacetylchitobiose. The overall structure shows a two-domain joined by a hinge-bending sugar binding region, which is similar to the maltose binding protein MalE of *Escherichia coli* and other solved sugar-binding protein of ABC transporter.

Keywords: ABC transporter, solute-binding protein, *N*-acetylglucosamine

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Crystal Structure and Structural Stability of Acylphosphatase from Hyperthermophilic Archaea *Pyrococcus horikoshii* OT3

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Pyrococcus horikoshii OT3 is a hyperthermophilic archaea that grows at temperatures between 88 ºC and 104 ºC. Proteins produced by this archaea possess high thermostability. To elucidate the structural basis for the high stability of acylphosphatase (AcP) from *P. horikoshii* OT3, we determined its crystal structure at 1.72 Å resolution. *P. horikoshii* AcP possesses high stability despite its approximately 30% sequence identity with eukaryotic enzymes that have moderate thermostability. Comparison with the crystal structure of eukaryotic AcP revealed some significant characteristics in *P. horikoshii* AcP that likely play important roles in structural stability: (i) shortening of the flexible N-terminal region and long loop; (ii) an increased number of ion pairs on the protein surface; (iii) stabilization of the loop structure by hydrogen bonds. In *P. horikoshii* AcP, two ion pair networks were observed, one located in the loop structure positioned near the C-terminus, and other on the β-sheet. The importance of ion pairs for structural stability was confirmed by site-directed mutation and denaturation induced by guanidium chloride.

Keywords: structure and stability of protein, thermostable, X-ray crystallography