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The penta-modular cellulosomal arabinoxylanase structure by x-ray crystallography and saxs

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The plant cell wall is one of largest repository of intractable and fixed carbon biosource on earth. It comprises myriads of interlocking polysaccharides displaying a high physical and chemical complexity. Thus, a very large repertoire of enzymes is needed for its total degradation. Certain microorganisms have evolved a highly elaborate, megadalton, extracellular multi-enzyme complex of cellulases and hemicellulases, termed the cellulosome, to carry out this biological conversion from complex polysaccharides to simple monosaccharides efficiently. The cellulosomal enzymes are multimodular with a variable architecture and size. However, each has a dockerin (DOC) module, which allows it to be integrated into the cellulosome by interacting with the cohesion module (COH) on the protein scaffold. The *Clostridium thermocellum* protein scaffold, CipA, has nine COH modules. Thereby, allowing any nine of the 72 dockerin-containing proteins to be incorporated. One such enzyme is the xylanase *Ct*Xyl5A (Cthe_2193), a multimodular arabinoxylanase that is one of the largest components of *C. thermocellum* cellulosome. *Ct*Xyl5A N-terminal catalytic domain, a glycoside hydrolase family 5 (GH5) member, is responsible for the hydrolysis of arabinoxylans (chemically and structurally complex polysaccharides comprising a backbone of β-1,4-xylose residues decorated with arabinofuranose (Ara*f*) moieties). Appended after it are three non-catalytic carbohydrate binding modules (CBMs), which belong to families 6 (CBM6), 13 (CBM13) and 62 (CBM62). The structure of the N-terminal bi-modular *Ct*GH5-CBM6 component showed that *Ct*GH5 displays a canonical $(\alpha/\beta)_8$-barrel fold with the substrate binding cleft with a tight hydrophobic interaction with the CBM6 [1]. CBM62 binds to D-galactose and L-arabinopyranose and mediates calcium-dependent oligomerisation [2]. *Ct*Xyl5A has a fibronectin type III-like (Fn3) module preceding the CBM62 [3] and following it, a type-I dockerin (DOC) module. We have obtained crystals of the penta-modular enzyme, excluding the DOC module at the C-terminal, with the architecture: *C*tGH5-CBM6-CBM13-Fn3-CBM62 [4]. The structure of this xylanase has been determined by Molecular Replacement using the *Ct*GH5-CBM6, Fn3 and CBM62 pdb coordinates to a resolution of 2.64 Å. The CBM13 module was built *de novo*. It displays a classic β-trefoil fold with an unusual track of 8 close tryptophan residues in one motif. Overall this 93 KDa penta-modular protein displays a compact structure for the first four modules with greater flexibility for the CBM62. This result has been corroborated with SAXS data.

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Crystal structure and nucleotide bound states of V_1-ATPase

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V-type ATPases (V-ATPases) belong to the rotary ATPase/synthase superfamily together with F-type ATPases (F-ATPases). Both enzymes work through the rotary catalytic mechanism for the ATP synthesis or hydrolysis. V-ATPases occur in the membranes of acidic organelles in eukaryotic cells, maintaining acidic pH by pumping protons coupled to ATP hydrolysis. On the other hand, they are also found in the plasma membranes of archaea and some eubacteria and these prokaryotic V-ATPases are primarily responsible for ATP synthesis, instead of F-ATPases.

Recent analyses of rotation kinetics of V-ATPase have revealed some differences between V- and F-ATPase in the generated torque and rotation steps. Therefore, the rotation mechanisms of V-ATPase and F-ATPase will be different from each other. The abundant studies for F-ATPase, including its crystal structures, have been reported and the rotation mechanism of F-ATPase is discussed in detail. In contrast, rotation mechanism of V-ATPase is poorly understood due to the lack of the structural information about complex state of the enzyme.

We have determined crystal structures of whole V_1-ATPase complex [1], which is the water-soluble component of V-ATPase and contain the active sites of ATP synthesis/hydrolysis. The structures were determined as nucleotide-free and nucleotide-bound forms at 4.8 and 4.5 Å resolutions, respectively, from thermophilic eubacterium, *Thermus thermophils*. The subunit composition for *Thermus* V_1-ATPase is A_3B_3DF and the nucleotide binding sites are located on the A subunits. The overall shape of V_1-ATPase is similar to that of F_1-ATPase. It consists of a cylindrical A_3B_3 hexamer and a central stalk composed of the D and F subunits penetrating the hexamer ring. However, significant differences in the conformation or structural motif between V_1- and F_1-ATPases are observed. In particular, the D subunit, which is the main component of the central stalk in V_1-ATPase, forms a long coiled-coil but shows apparently more straight conformation than the corresponding γ subunit of F_1-ATPase. This conformational difference can explain the variations of the generating torque of both enzymes.

In the nucleotide-bound form of V_1-ATPase, nucleotides bind to the two of three A subunits but the ternary changes are scarcely observed among the three subunits. In contrast, significant quaternary rearrangements are observed around nucleotide binding sites located at the interfaces of the A and B subunits. These quaternary structures are almost the same as those of F_1-ATPase. Therefore, the common property between V_1- and F_1-ATPases is only on the structural arrangement of the subunit interfaces around the active sites, strongly suggesting that the rotation of V_1-ATPase is primarily driven by the quaternary changes around the interface of nucleotide binding sites. We have investigated further structural analyses for the complex of some nucleotide analogs reveal more detailed information for the nucleotide bound states of V_1-ATPase.

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Structural analysis of MamA, a magnetosome associated protein from two different magnetospirillium species

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Tetra- tricopeptide repeat (TPR) is a structural motif found as such or forming part of a bigger fold in a wide range of proteins. It serves as a template for protein-protein interactions and mediates multiprotein complexes [1]. MamA is a unique, highly abundant, Magnetosome associated protein and predicted to contain 5 TPR motifs as well as predicted putative one. Magnetosome is a subcellular organelle that consists of a linear-chain assembly of inner membrane invaginations each able to biomineralize and enclose a ~50-nm crystal of magnetite or greigite. Magnetosome allows magnetotactic bacteria, a diverse group of aquatic microorganisms, to orientate themselves along geomagnetic fields in search of suitable environments [2]. MamA is one of the most characterized magnetosome-associated proteins *in vivo* and yet, its function is not clear [3-5]. Here, we report on the crystallization and structure analysis of recombinant *M. magneticum* (AMB-1) and *M. gryphiswaldense* (MSR-1) MamA deletion mutants. The structures were determined to a resolution of 2.0 Å and confirmed MamA fold as a five TPR motifs containing protein.

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The mechanisms of self-assembly of the vault, the largest cytoplasmic ribonucleo-protein complex

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Vaults are the largest cytoplasmic ribonucleo-protein particles found in numerous eukaryotic species. They were first observed in 1986 as contaminants in preparations of clathrin-coated vesicles from rat liver. Rat liver vault comprises three kinds of proteins: the major vault protein (MVP) , the vault poly(ADP-ribose) polymerase (VPARP) , the telomerase- associated protein 1 (TEP1) and a small untransrated RNA consisting of 141 bases (vRNA). The mass of rat liver vault is about 10 MDa, and the particle shell measures about 700 Å in length and about 400 Å in maximum diameter. Most vault particles are present in the cytoplasm, but few of them (about 5% of the total vault fraction) localize to the nucleus. Several studies suggested that vaults might play an important role in the multi-drug resistance (MDR) of cancer cells. Human vRNAs have the ability to bind mitoxantrone, a chemotherapeutic compound, and they may play an important role in the export of toxic compounds (Gopinath et al., Nucleic Acids Res., **33**, 4874-4881 (2005)). The recent study shows that vaults may be involved in innate immunity (M. P. Kowalski et al., Science **317**, 130-132 (2007)). However, their cellular function remains unclear.

We have determined the x-ray structure of rat liver vault at 3.5Å resolution in 2008 [1, 2]. X-ray structure reveals that vault particle has 39-fold dihedral symmetry and shell is made up of 78 identical MVP chains. Each MVP monomer folds into 12 domains: nine structural repeat domains, a shoulder domain, a cap-helix domain, and a cap-ring domain. Side-by-side hydrophobic interactions of the cap-helix domain play a key role for self-assembly of the half-vault. N-terminal residues of MVP domain 1 form intermolecular interactions around two-fold axis including anti-parallel sheet and ionic bond. In this session, we will discuss the mechanisms of self-assembly of the vault.

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Modeling the organization of molecules in collagen using the paracrystal concept

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A characteristic feature of the dense phases formed by fiber-shaped molecules is their organization into parallel rods packed in a hexagonal or pseudo-hexagonal lateral network. This is typically the case for the collagen triple helices inside fibrils, as confirmed by recent X-ray diffraction experiments carried out on highly crystallized fibers obtained by immersing the freshly extracted fibers in a salt-controlled medium.

However such diffraction patterns also generally exhibit additional features in the form of diffuse scattering, which is a clear signature of a low degree of lateral ordering. Only few studies have analyzed and modeled the lateral packing of collagen triple helices when the structure is disordered. Some authors have used the concept of short-range order but this approach does not contain any echo of a hexagonal order. In this study [1], we use an analytical expression derived from the paracrystal model which retains the hexagonal symmetry information and leads to a good agreement with the experimental data in the medium-angle region. This method is quite sensitive to the degree of disorder and to the inter-object distance. *One clear result is that the shift in peak positions, generally attributed to variations in intermolecular distances, can also arise from a change in the degree of ordering without any significant modification of the distances*. This underlines the importance of evaluating the degree of ordering before attributing a shift in peak position to a change in the unit-cell. This