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Keywords: Bioenergetics, F-type ATP synthase, Molecular motor

MS08.P04

Acta Cryst. (2011) A**67,** C263

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

Shabir Najmudin,[a] Joana L. A. Brás,[a] Adam R. Round,[b] José A. M. Prates,[a] Maria J. Romão,[c] Harry J. Gilbert,[d] Carlos M. G. A. Fontes,[a] *[a]CIISA - Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, Lisboa, (Portugal).[b]EMBL Grenoble,6 rue Jules Horowitz, BP 18, 38042 Grenoble, Cedex 9, (France),[c]REQUIMTE, Departamento de Química, FCT-UNL, Caparica, (Portugal).[d]Institute for Cell and Molecular Biosciences, Newcastle University, The Medical School, Newcastle upon Tyne NE2 4HH, (UK).* E-mail: shabir@fmv.utl.pt.

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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Keywords: arabinoxylanase, cellulosome, plant cell wall degradation

MS08.P05

Acta Cryst. (2011) A**67,** C263-C264

Crystal structure and nucleotide bound states of V_1-ATPase

Nobutaka Numoto,[a] Takeshi Kuranaga,[b] Yumemi Nagamatsu,[b] Yu Hasegawa,[b] Akiko Kita,[a] Kazuki Takeda,[b] Kunio Miki,[b] *[a]Research Reactor Institute, Kyoto University, Kumatori, Osaka. [b]Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto (Japan).* E-mail: numoto@kuchem.kyoto-u.ac.jp

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.