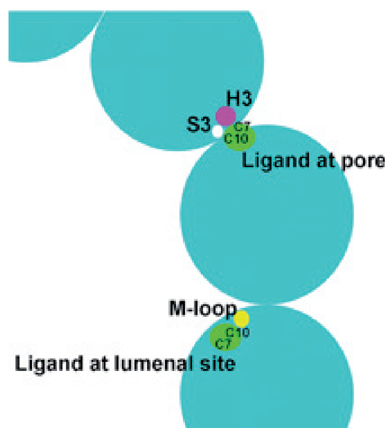
docking techniques and molecular dynamics simulations. Modeling results indicate that positions C7 and C10 affect the conformation of three key elements (the M-loop, S3 and H3) in the lateral interactions that modulate the contacts between adjacent protofilaments. Alternatively, the change in C2 slightly rearranges the ligand in the binding site, thus modifying the interaction of the ligand C7 position with the M-loop.



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Scheme of the interactions of taxane ligands at the pore and luminal binding sites of microtubules, with the structural elements responsible for the interprotofilament interaction. The microtubule protofilaments are seen from the plus end

Key words: SAXS, protein, assembly, NMR, molecular dynamics

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A redox-controlled synergistic mechanism regulates the binding of the intrinsically disordered protein CP12 to photosynthetic glyceraldehyde-3-phosphate dehydrogenase

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Carbon assimilation in plants is regulated by the reduction of specific protein disulfide bridges by light and their re-oxidation in the dark [1]. The redox switch, CP12, is a small, intrinsically-disordered protein that carries two disulfides groups. In the dark, it forms an inactive supramolecular complex with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [2],[3]. Here we show that binding of CP12 to GAPDH follows a synergistic mechanism that includes both conformational selection and induced folding steps. Initially, a conformation characterized by a circular structural motif of the C-terminal disulfide of CP12 is selected by GAPDH. Subsequently, the induced folding of the flexible C-terminal tail of CP12 in the active site of GAPDH site stabilizes the binary complex. Formation of several hydrogen bonds compensates the entropic cost of CP12 fixation and terminates the synergistic mechanism that controls carbon assimilation.

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Rotor architecture in the yeast F_1-c_{10}-ring complex of F-ATP synthase

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F_1F_o-ATP synthase is a hybrid molecular nanomotor. F_1 is a chemical motor driven by ATP hydrolysis while F_o is an electrical motor driven by the proton flow. The two stepping motors are mechanically coupled through a common rotary shaft. The F_1-ATPase X-ray structures support binding change mechanism for catalysis. The empty and open catalytic site β_E is the OPEN site with low affinity for nucleotides, the β_{TP} site filled by ATP (or AMP-PNP) is the LOOSE conformation and the β_{DP} site filled by ADP is the TIGHT conformation where synthesis occurs. In F_1 domain, the orientation of the γ-subunit relative to the $(\alpha\beta)_3$ component determines the catalytic state of the enzyme and, according to the binding change mechanism, a 120° rotation of the γ-subunit during the ATP hydrolysis cycle results in E→TP, TP→DP and DP→E interconversions.

Up to now, the three available crystal structures of the F_1c_{10} sub-complex of the yeast F_1F_o-ATP synthase were isomorphous [1] and [2] with a crystal form named yF_1c_{10}(I).

In the crystal structure of the Mg.ADP-inhibited state of the yeast F_1c_{10}-ATP synthase, solved at 3.4 Å resolution, an ADP molecule was bound in both β_{DP} and β_{TP} catalytic sites [1]. The α_{DP}-β_{DP} pair is slightly open and resembles the novel conformation identified in the yeast F_1 [3], whereas the α_{TP}-β_{TP} pair is very closed and resembles more a DP pair. In the F_o rotor ring, the essential cGlu59 carboxylate group is only surrounded by apolar residues. Its closest hydrogen bond acceptor, the cLeu57 carbonyl oxygen of the adjacent c-subunit, is too far away to make a direct hydrogen bond. The proton binding has specific features compared to the bacterial Na^+-transporting or the cyanobacterial and chloroplastic H^+-transporting F-type ATP synthase rotor structures. In the crystal, significant interactions of the c_{10}-ring with the F_1-head of neighboring molecules affect the overall conformation of the F_1-*c*-ring complex. The symmetry axis of the F_1-stator and the inertia axis of the *c*-ring are tilted near the F_1-F_o rotor interface, resulting in an unbalanced machine.

Recently, we have solved a new crystal form of the yeast *Saccharomyces cerevisiae* F_1c_{10} complex, named yF_1c_{10}(II), inhibited by adenylyl imidodiphosphate (AMP-PNP) and dicyclohexylcarbodiimide (DCCD), at 6.5 Å resolution in which the crystal packing has a weaker influence over the conformation of the F_1-*c*-ring complex.

Despite a low resolution, the overall fold is clearly visible with a more straight C-terminal helix of subunit γ. Though the F_1-stator is 8° tilted relative to the rotor axis, its center of mass is located approximately on this axis. Therefore, yF_1c_{10}(II) provides a model of a more efficient generator. The present yeast yF_1c_{10}(II) and the bovine bF_1c_8 [4] models are comparable and together provide accurate models of the F_1-*c*-ring domain in the intact F_1F_o-ATP synthase.

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

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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.