Diversity in P-loop Structure of A-ATP Synthase

ATP synthase and ATPase are the key components in energy utilization in cells. They are essentially the turbines in powerhouse to create energy in the form of ATP or use ATP as energy to drive cellular activities. P-loop is a common motif in ATP and GTP binding proteins, including ATP synthase and ATPase, and its conformation change is critical in A/GTP utilization. Prof. Grüber’s group solves several structures of A-ATP synthases. It contributes to our knowledge of P-loop. Differences as small as 3 amino acids in the P-loop sequence change its conformation and have a profound impact on its effectiveness. It demonstrates how nature optimizes biological activities down to such tiny details.

A-, F-ATP synthases and V-ATPases are fascinating enzymes, which arose from a common ancestor and are present in every life form. They are essential for life and are known as the coupling factors which convert the electrochemical ion gradient across the membrane to the synthesis of adenosine triphosphate (ATP) and vice versa. F,Fo ATP synthases (F-ATP synthases) are responsible for the generation of ATP in the eukaryotic and prokaryotic cells, while in archaea the A1A0 ATP synthases use the H+- and/or Na+-gradient across the membrane to drive ATP production. Largely ATP synthases use the ion motive force for generating ATP, whereas the vacuolar ATPases (V-ATPase) of eukaryotic cells hydrolyze ATP to generate proton motive force. The variations in the subunit composition drive the difference in their functions. The A-ATP synthase is composed of nine subunits in a proposed stoichiometry of A3 : B3 : C : D : F : H2 : α : c, while the related bacterial F-ATP synthase has eight subunits (α3 : β 3 : γ : δ : ε : a : b 2 : c x) and the eukaryotic V-ATPase has thirteen subunits (A3 : B3 : C : D : E : F : G2 : Hx : α : d : c : c′ : c″).

The A-ATP synthase is composed of two functional sectors; a water soluble A1 part, containing the catalytic sites, and the membrane bound A0 sector, involved in ion translocation. Both of these domains are connected through the stalk part containing subunits C-F, H and α. Subunit A has been regarded as having catalytic function while subunit B plays an important role in nucleotide binding and/or regulatory function. Crystallographic structures of the nucleotide-binding subunits A and B of A-ATP synthases show that they are composed of an N-terminal β barrel, an α-β central domain, and a C-terminal α-helical bundle with the exception of A-subunit having an additional domain called non-homologous region (NHR), an insertion of about 90 amino acids near the N-terminus. Although the consensus sequence (GXXXGKT), called phosphate binding loop (P-loop), is present in the catalytic A subunit of A-ATP synthases (GPFGSGKT), V-ATPases (GAFGCGKT) and subunit β of F-ATP synthases (GGAGVGKT) they differ in sequence composition, implying diversities in nucleotide recognition and/or catalytic mechanism.
Most recently, transition position of ADP and ATP could be described in the crystallographic structures of subunit B, providing information on the ATP traversing pathway to the final binding pocket. However, the mechanism of nucleotide-binding and ATP synthesis in subunit A of A-ATP synthases still remains a puzzle. Here we describe the crystal structure of subunit A from P. horikoshii OT3 A-ATP synthase in the absence (A$_E$) and presence of AMP-PNP (A$_{AMP}$) (Fig. 1(a)) as well as ADP (A$_{ADP}$) at 2.47 Å and 2.4 Å resolutions. In the A$_E$ structure the phosphate analog SO$_4^{2-}$ is bound via a water molecule to the P-loop residue, S238. The sulfate ion is located at a position between the β- and γ-phosphate of the AMP-PNP molecule. Although crystallized under identical conditions, no sulfate ion is present in the mutant S238A structure, implying that the A$_E$ structure may represent an intermediate state of the Pi-binding site. The importance of S238 in phosphate binding is emphasized by hydrogen bonding of S238 with α- and β-phosphates of ADP via a water molecule, and the bifurcated hydrogen bond with the γ-phosphate of AMP-PNP in the A$_{AMP}$ structure, respectively (Fig. 1(b)). The γ-phosphate of AMP-PNP is now bridged by a water molecule to P235, which moves out of the active site, when ADP is bound, opening thereby the site for the ADP + Pi binding (Fig. 1(c)).

When compared to the related F$_1$F$_0$ ATP synthase of the rat liver mitochondrial, P235 is the neighbouring amino acid residue of A158 in the P-loop of the catalytic subunit β and proposed to be critical in the final events of ATP synthesis.

Structural overlap of the AMP-PNP bound β-subunits (β$_{AMP}$) of bovine F$_1$-ATPases with the A$_{AMP}$ structure clearly demonstrate that the novel P-loop conformation in subunit A forces the nucleotide into a different arrangement inside the catalytic site with weaker interactions of different and/or homologue surrounding amino acid residues and making the nucleotide more solvent exposed. This may explain the ability of A-ATP synthases to hydrolyze apart from ATP also GTP and UTP and may explain the similar binding constants of subunit A for MgATP and MgADP. By contrast, a preference for the ATP over the ADP analogue has been measured for subunit β of F-ATP synthases as well as subunit B of the A-ATP synthase, whose P-loop arrangement is similar to the one of subunit β.

Surprisingly when the S238A mutant of A is compared with the bovine β$_{E}$, the P-loop conformation of both the structures exactly matches each other, indicating a significant role played by S238 in the loop conformation of subunit A. A closer look to the P-loop environ-
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Fig. 2: The polar interaction profile of the P-loop region (green) in the empty form of the A subunit. The network of polar interaction (black dotted lines) stabilizing the P-loop is indicated.

In order to analyze and understand the role of each amino acids in the P-loop region in more detail, the alanine mutants of P235 and F236 were generated and their crystal structures were determined to resolutions of 2.38 Å and 2.35 Å, respectively. The structures display novel conformations for the P-loop, which represent the intermediate states in between the fully arched (wild type) and well relaxed (S238A) conformations, directly indicating the important role played by them in the catalytic pathway (Fig. 3). Even though the deviation is similar for both mutants the curvature of the P-loop faces opposite direction. Two major deviations were observed in the P235A mutant, which are not identified in any of the subunit A structures analyzed so far. One being a wide movement of the N-terminal loop region (R90-P110) making a rotation of 80° and the other is the rigid body rotation of the C-terminal helices from Q520-A588 by around 4° upwards. This movement is attributed to the difference in the interaction profile that holds the P-loop and the C-terminal helices Q520-A588 between the P235A mutant and the wild type. In the P235A mutant structure T513 interacts with W250, which is missing in wild type and this W250 is placed near to the loop region 252DAQ254, which is the structural equivalent position of the hinge region (177HGG179) of subunit β, known to be involved in the C-terminal domain movement in F-ATP synthases. Therefore, it could be postulated that the T513 in subunit A can act like a switch in coupling the P-loop disturbances, due to the mutation, to the C-terminal helical region, making a conformational change by about 4° rotation.
The crystallographic structures presented show that the alterations in the three critical residues in the P-loops of catalytic A subunit (GPFGSGKT) cause the conformational rearrangement of the arched P-loop in subunit A into a relaxed form as in the β subunit of F-ATP synthases. This evolutionary switch in amino acid sequence and conformation of P-loops resulted in altered nucleotide-binding, -specificity, and -accessibility as well as catalytic variation in the energy producers A- and F-ATP synthase. As the P-loop is essential for enzymatic processes of ATP synthesis and -hydrolysis, catalyzed by the energy producers A- and F-ATP synthases and the V-ATPase, the observed diversity in P-loop structure are a milestone in understanding the different mechanism and regulation in these three classes of engines.

**References**


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