Nucleotide-binding States of Subunit A of the A-ATP Synthase and the Implication of P-loop Switch in Evolution

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Archaeal ATP synthases are energy providing machines, converting adenosine diphosphate (ADP) and inorganic phosphate to adenosine triphosphate (ATP). The multi subunit complex is composed of two domains: a water soluble A_1 and the membrane bound A_O domain. The ATP synthesis is carried out in the A_1 domain which consists of subunits A and B in a hexameric arrangement [1]. Subunit A has been regarded as having catalytic function while subunit B has either binding or regulatory function. The crystal structure of the nucleotide free subunit A [2] and B [3] reveled that although the overall structure are similar containing the three domains, an N-terminal β barrel, an central α-β domain, and a C-terminal α-helical bundle, subunit A has one another domain termed non-homologous region (NHR) in between the N-terminal β barrel and the central α-β domains whose functional relevance is yet to be determined. And subunit A has the typical nucleotide binding motifs, Walker A (also called as P-loop) and B, which is absent in B subunit. But it has been shown by photoaffinity labelling and fluorescence correlation spectroscopy [3,4] that B-subunit binds to MgATP and –ADP and that it contains the loop similar to P-loop with the sequence $_{150}$SASGLPHN$_{157}$. Most recently, transition position of ADP and ATP could be described in crystallographic structures of subunit B, providing information on the ATP traversing pathway to the final binding pocket [4-6]. However, the mechanism of nucleotide-binding and ATP synthesis in subunit A of A-ATP synthases still remains a puzzle.

In our attempt to understand the nucleotide binding ability of A-subunit, we have successfully developed protocols to co-crystallize the A-subunit of the A_1A_O ATP synthase from *Pyrococcus horikoshii* OT3 in complex with AMP-PNP (A_{PNP}) (5'-adenylyl-β,γ-imidodiphosphate) and ADP (A_{DP}) bound forms as well as in the nucleotide-empty (A_E) form [7]. The structures solved at 2.47 Å and 2.4 Å resolutions, respectively, provide novel features of nucleotide-binding and depict the residues involved in catalysis of A. In the A_E form, the phosphate analogy SO_4^{2-} binds via a water molecule to the P-loop residue Ser238, which is also involved in phosphate-binding of ADP and AMP-PNP. Together with the amino acids Gly234 and Phe236, the serine residue stabilizes the arched P-loop conformation of subunit A as shown by the 2.4 Å structure of the mutant protein S238A, in which the P-loop flips into a relaxed state, comparable to the one in catalytic β subunits of F-ATP synthases. Superposition of existing P-loop structures of ATPases emphasize the unique P-loop in subunit A suggesting an important evolutionary switch in P-loop and thereby in nucleotide recognition and mechanism of ATP synthesis and/or ATP hydrolysis of the biological machines A-, F-ATP synthases and V-ATPase[7].

We have also attempted to mutate the important residues involved in nucleotide binding of the A-subunit and to co-crystallize with different nucleotides. Data for many of them have been collected and currently structure refinement for the P-loop mutants are going on.

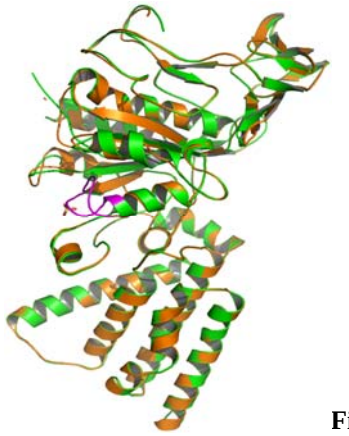

Fig. 1: Structural comparison of empty (orange) and S238A mutant (green) structures. The P-loop highlighted in magenta shows a momentous deviation.

References

[1] V. Müller and G. Grüber, Cell Mol. Life Sci. **60**, 474 (2003).
[2] Y. Maegawa, H. Morita, D. Iyaguchi, M. Yao, N. Watanabe, and I. Tanaka, Acta. Cryst. D **62**, 483 (2006).
[3] I. Schäfer, S. M. Bailer, M. G. Düser, M. Börsch, A. B. Ricardo, D. Stock, and G. Grüber, J. Mol. Biol. **358**, 725 (2006).
[4] A. Kumar, M. S. S. Manimekalai, A. M. Balakrishna, C. Hunke, S. Weigelt, N. Sewald, and G. Grüber, Proteins **75**, 807 (2009).
[5] M. S. S. Manimekalai, A. Kumar, A. M. Balakrishna, and G. Grüber, J. Struct. Biol. **166**, 38 (2009).
[6] A. Kumar, M. S. S. Manimekalai, and G. Grüber, Acta Cryst D **64**, 1110 (2008).
[7] A. Kumar, M. S. S. Manimekalai, A. M. Balakrishna, J. Jeyakanthan, and G. Grüber, J. Mol. Biol. **396**, 301 (2010).