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S1.P10

The ingenious structure of central rotor apparatus in V_oV_1: Key for both complex disassembly and energy coupling between V_1 and V_o

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The vacuole-type ATPases (V_oV_1) are found in many organisms and are involved in a variety of physiological processes. The V_oV_1 and F_oF_1 ATPases/synthases are evolutionarily related and share a rotary mechanism to perform their specific functions. The basic structures of the ATPases/synthases are conserved among species. The soluble, cytoplasmic portion of F_oF_1 and V_oV_1 (called F_1 and V_1, respectively), responsible for ATP hydrolysis/synthesis, is connected via the central shaft and the peripheral stator stalk to the transmembrane portion (F_o and V_o) that houses the ion transporting pathway. In F_oF_1, a central shaft, γ-subunit, binds directly to the rotor ring. In contrast, at the boundary surface of V_oV_1, V_o-C forms a socket-like structure which accommodates the V_1-DF central shaft, indicating that V_1-DF does not contact the rotor ring directly. Thus, the boundary surface of V_oV_1 is significantly different from that of F_oF_1. In this study, reconstitution and fluorescence resonance energy transfer analysis of V_oV_1 subcomplexes revealed a weak binding affinity of V_1-DF to V_o-C despite the fact that torque needs to be transmitted between V_1-DF and V_o-C for the tight energy coupling between V_1 and V_o. Mutation of a short helix at the tip of V_1-DF caused intramolecular uncoupling of V_oV_1 and a decrease in binding affinity of the mutated V_1 for V_o, suggesting that proper fitting of the short helix of V_1-D into the socket of V_o-C is required for tight energy coupling between V_1 and V_o. To account for the apparently contradictory properties of the interaction between V_1-DF and V_o-C (weak binding affinity but strict requirement for torque transmission), we propose a model in which the relationship between V_1-DF and V_o-C corresponds to that between a slotted screwdriver and a minus thread screw. This model is consisted of our previous result in which the central rotor apparatus is not the major factor for the association of V_1 with V_o.

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Molecular basis of ADP-inhibition of V type ATPase/synthase

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Vacuolar type ATPase (V_oV_1) functions as an ATP hydrolysis-driven proton pump that carries out acidification of cellular compartments in eukaryotes. A family of V_oV_1, sometimes referred to as the A-type ATPases or A_oA_1, is also found in archaea and some eubacteria (the prokaryotic V_oV_1 family). In most prokaryotes, such as *Thermus thermophilus*, the V_oV_1 functions as an ATP synthase. However it can also act as a primary ion pump, as seen in *Enterococcus hirae*.

ADP-inhibition caused by entrapment of ADP at a catalytic site is believed to be a regulatory mechanism of F_oF_1 to prevent wasteful ATP consumption when proton motive force is lost. V_oV_1 from *T. thermophilus* (*Tth*V_oV_1) also exhibits sensitivity to ADP inhibition, resulting in rapid decay of the ATPase activity of the V_1 subcomplex. Contrary, V_oV_1 of *E. hirae* or eukaryotes show no sensitivity to ADP-inhibition, relevant to the physiological role of this ion pump. ADP-inhibition has been investigated in F_oF_1, but the precise molecular mechanism remains poorly understood.

To investigate the molecular basis for this difference, domain swapped chimeric V_1 consisting of both *T. thermophilus* and *E. hirae* enzymes was generated and its function analyzed. The data showed that the interaction between the nucleotide binding and C terminal domains of the catalytic A subunit from *E. hirae* V_1 is central to increasing binding affinity of the chimeric V_1 for phosphate, resulting in reduction of the ADP-inhibition. These findings together with a comparison of the crystal structures of *T. thermophilus* V_1 with *E. hirae* V_1 strongly suggest that the A subunit adopts a different conformation in *T. thermophilus* V_1 from that in *E. hirae* V_1. This key difference results in ADP inhibition of *T. thermophilus* V_1 by abolishing the binding affinity for phosphate during ATP hydrolysis.

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S1.P12

The effect of betaq259l mutation on MgADP-inhibition of *B. subtilis* H^+-F_OF_1-ATP-synthase

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The ATPase activity of H^+-F_OF_1-ATP synthase can be regulated in several ways, but the most common of them is inhibition by MgADP. It is known for all studied F_OF_1 complexes that MgADP bound in a catalytic site in the absence of phosphate may cause enzyme inactivation instead of being released and replaced by new MgATP. The degree of this inhibition varies greatly between different organisms: the inhibition is strong in chloroplast F_OF_1 and enzymes of some bacteria (*Bacillus* PS3, *Bacillus subtilis*), but in F_OF_1 of *Escherichia coli* it is much weaker.

It has been reported previously [1] that a point mutation Gln259Leu in the beta subunit of *Bacillus* PS3 F_OF_1 noticeably relieves its strong MgADP inhibition. In this work, we inserted the same mutation in *B. subtilis*. MgADP inhibition in our mutant F_OF_1 also weakened significantly in comparison to the wild-type enzyme. The mutant was capable of ATP-driven proton pumping and was sensitive to azide like the wild type. The initial ATPase activity levels of the mutant and of the wild-type F_OF_1 complexes were comparable at room temperature and at 37 °C, but the mutant enzyme has demonstrated sensitivity to cold temperatures. The living cells of the mutant strain also were not resistant to cold. To clarify the physiological role of MgADP inhibition in *B. subtilis*, we also compared wild-type and mutant strains in competition growth experiments under different conditions.

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