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S1.P7

The role of residue beta249 in MgADP inhibition of *Escherichia coli* ATP synthase

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When MgADP without phosphate is bound at a catalytic site of H^+-FoF1 ATP synthase, there is a probability that the enzyme gets blocked in an inactive conformation. This phenomenon is known as MgADP inhibition. Earlier experiments indicate that the enzyme adopts a special inactive conformation with MgADP trapped in a catalytic site. This is a core feature of ATP synthase observed even in the minimal catalytic alpha3beta3gamma-complex. MgADP inhibition is found in all ATP synthases studied so far. However, the degree of inhibition varies considerably, being strong in mitochondria and especially chloroplast enzyme, as well as in some bacteria, e.g. *Bacillus* PS3, but rather weak in FoF1 from *Escherichia coli*. We have demonstrated previously that in *Bacillus* PS3 ATP synthase mutation betaGln259Leu can dramatically decrease MgADP inhibition. In *E. coli* wild type enzyme the residue in the corresponding position (beta249) is Leu, i.e. it is similar to the mutant *Bacillus* PS3 enzyme. In this work we made an *E. coli* mutant betaLeu249Gln. The mutation significantly enhanced MgADP inhibition. Moreover, it completely changed the effect of phosphate on MgADP inhibition, while in the wild-type enzyme phosphate increased the inhibitory effect of MgADP, and in the betaLeu249Gln it relieved the inhibition. We conclude that MgADP inhibition is a relatively easily modulated regulatory feature of ATP synthase rather than an inevitable "side effect" of the rotary catalytic mechanism.

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S1.P8

The Fo complex of the chloroplast ATP synthase — Towards a structural study of a macromolecular nanomotor

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Powered by the proton-motive force (pmf) of the cell, F-type ATP synthases are the major producers of adenosine triphosphate (ATP) in cells. The ATP synthase in plant chloroplasts consists of two domains, the soluble F1 and the membrane-embedded Fo subcomplex. In Fo the pmf across the thylakoid membrane is converted into torque and induces rotation of a rotor element, the c-ring. The unique construction of the Fo complex allows ions (H^+ or Na^+) to pass a distinct pathway through the rotor–stator elements and surmount the membrane from one side to another. The rotation is tightly coupled with F1 and elicits conformational changes in the catalytic beta-subunits, finally leading to ATP synthesis. While the function of the F1 complex and the Fo rotor element has unveiled by many high-resolution structures in the last 20 years, structural information about the complete Fo complex or its neighboring stator subunits a and b is still scarce. To understand the molecular mechanism of this nanomotor, high resolution structural information is indispensable. We set out to investigate the chloroplast Fo-ATP synthase complex by structural methods. By combining classical biochemical purification strategies such as sucrose density centrifugation and ion-exchange chromatography we were able to establish a protocol for a large scale purification of the ac14 sub-complex of the *Spinacia oleracea* ATP-synthase. The isolation of abundant and stable ac14 allows subsequent experiments such as 2D and 3D crystallization and their structural characterization by electron microscopy and X-ray crystallography.

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S1.P9

A theoretical treatment of energy transfer in a molecular motor based on Brownian motion on a multidimensional free-energy landscape: The derivation of formal analytic results in the Kramers regime of deep potential wells

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In a cell, specialised proteins act as molecular-scale motors to convert energy from one form to another. For example, ATP synthase converts chemical energy into mechanical work and back again, ion pumps convert chemical energy into transport against an electrochemical gradient, and cytoskeletal motors use chemical energy to transport loads along linear molecular chains. Despite functional differences between different types of molecular motors, there are fundamental similarities in their behaviour and properties: i) they transfer energy between different degrees of freedom (i.e., chemical and mechanical), ii) they are subject to large thermal fluctuations, and iii) they operate far from equilibrium. We present a general theoretical treatment of energy transfer in a molecular motor based on Brownian motion on a multidimensional free-energy landscape. This treatment enables energy transfer between degrees of freedom to be described explicitly and captures key fundamental elements of molecular motor operation. Unfortunately, the multidimensional diffusion equation arising in this treatment is not solvable in general. We transform the continuous diffusion equation to a simpler discrete master equation that is analytically tractable [1]. This provides an opportunity to connect the general continuous multidimensional Brownian-motion based theory with experiments, phenomenological models, and established results from non-equilibrium thermodynamics. In the Kramers regime of deep potential wells, we derive a range of formal expressions for molecular motors. We determine physical properties including the drift and diffusion, the rate and efficiency of energy transfer, and the entropy generated [2]. We also consider thermal fluctuations for the motor and show that energy transfer between degrees of freedom creates statistically correlated fluctuations in those degrees of freedom [3]. The fluctuation statistics provide an opportunity to distinguish different operating regimes of the motor.

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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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S1.P11

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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