S1.36 Solution structure of subunit *d*, E and G of the eukaryotic V-ATPase

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Vacuolar ATPases (V-ATPases) are abundant, ubiquitous proton pumps in eukaryotic cells. These pumps regulate pH and generate an electrochemical gradient that drives the transport of molecules across membranes of endosomes, golgi, secretory vesicles, vacuoles, lysosomes or plasma membrane of osteoclasts and kidney intercalated cells. Eukaryotic V-ATPases are composed of at least 14 different subunits in a stoichiometry of A_3:B_3:C:D:E_X:F:G_2:H_X:*a*:*c*:*c*′:*c*″:*d*:*e*. The enzyme is divided into V_1- and V_O parts. A critical point in V-ATPases is the structure of stalk subunits *d*, E and G. Here, we will present the first low resolution structure of subunit *d* of the *Saccharomyces cerevisiae* V_1V_O ATPase, determined from solution X-ray scattering data. The protein is a boxing glove-shaped molecule consisting of two distinct domains, with a width of about 6.5 nm and 3.5 nm, respectively. Furthermore, the 3D structure of E_{1-69} and G_{1-59} of subunit E and G, respectively, has been solved using NMR spectroscopy. Binding studies using ^{1}H–^{15}N heteronuclear single quantum coherence spectra of G_{1-59} show specific interactions only with the peptide E_{18-38} of subunit E and allow a clear assignment of interacting amino acids in the E–G assembly.

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S1.37 Changes in crosslink efficiency between *b* subunits in the peripheral stalk of F_1F_O ATP synthase

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Chimeric *b* subunits have been produced by substituting regions of the *b* and *b*′ subunits from the photosynthetic bacteria *Thermosynechococcus elongatus* into the *b* subunit of *Esherichia coli*. These chimeric subunits readily formed heterodimeric peripheral stalks when the region E39–I86 of the *E. coli* enzyme was replaced with *T. elongatus* sequences, producing chimeric subunits abbreviated *Tb* and *Tb*′. Cysteines were substituted for A83 or A90 in both *Tb* and *Tb*′ subunits and disulfide crosslink formation was used to probe for relative subunit position. Crosslinks formed readily between both Tb_{A83C}/Tb'_{A90C} and Tb_{A90C}/Tb'_{A90C} when the enzyme was at rest. However, addition of ATP greatly reduced the efficiency of crosslink formation. Additional constructs were made which expressed normal *E. coli b* subunit with cysteine substitutions at several positions. F_1F_O F_1F_0 ATP synthase with homodimeric $(b_{A90C})_2$ showed a similar ATP-dependent inhibition of crosslink formation while complexes with $(b_{I76C})_2$ and $(b_{R83C})_2$ did not.

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S1.38 Expression vectors for mutant peripheral stalk subunits in *Escherichia coli* and *Saccharomyces cerevisiae*

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The function of the peripheral stalk of F_1F_O ATP synthase is to hold the F_1 sector against the movement of the rotor stalk during catalysis. The aim of this study was to generate expression vectors to investigate the capacity of *E. coli* and *S. cerevisiae* peripheral stalk subunits to tolerate changes in length. *E. coli* plasmids containing the entire *unc* operon that included insertions and deletions in the peripheral stalk were constructed. ATP driven proton pumping assays in isolated membrane vesicles indicated approximately 50% of wild type activity. The eleven amino acid insertion was significantly more active than our previous studies with the mutant *b* subunit expressed individually from a plasmid. Examination of the high-resolution structure of the bovine peripheral stalk from the Walker laboratory suggested a site in the eukaryotic enzyme peripheral stalk that might accept insertions and deletions. Expression plasmids have been constructed for the wild type and an insertion into a synthetic ATP4 (*b* subunit) gene of *S. cerevisiae*. These constructs will be used to investigate the structure–function relationships within the eukaryotic peripheral stalk.

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S1.39 Quantum-mechanical approach to the description of proton diffusion in the membrane protein pore of ATP-synthase

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Diffusion of protons is a well-known key process in the coupling of oxidative phosphorylation in mitochondria. Nevertheless, the molecular mechanisms of H+ movement inside the protein pore remain still incomprehensible. We try to describe these processes using a quantum-mechanical model which can help us to estimate average time of both a proton jump from essential protonated residue (cAsp61) away to membrane surface and reverse movement as well. For this aim the internal space of each half-channel was approximated as a parallelepiped area which included all essential residues. Then wave functions were determined as the solutions of steady state Schrödinger equation for centrally symmetric field of pulse charge system. The localization of protons within the channel was assumed to be near oxygen or nitrogen atoms of both protein residues and water. It was shown that there were several ways for proton trace in the channel area and these traces had different types of energy emission. So we suppose that this difference can be the base for understanding of molecular mechanism of energy transformation of electro-chemical gradient into the energy of ATP bond.

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S1.40 Domain motions of the catalytic subunit β in F_1-ATPase revealed by single molecule observation

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The aim of the present study is to understand the mechanism of converting chemical energy into mechanical work in the rotary molecular motor F_oF_1-ATP synthase. Conformational changes of the catalytic subunit β which are assumed to drive rotation of the central stalk γ are the key to solve this question. In order to investigate them, we have applied single molecule TIRF microscopy with polarisation modulation to observation of $\alpha_3\beta_3\gamma$ subcomplex of F_oF_1-ATP synthase. Simultaneous but independent observation of conformational changes of β and rotational substeps of γ in single $\alpha_3\beta_3\gamma$ molecules revealed a sequence of conformational changes of the β subunit during ATP hydrolysis between "Open", "Closed", and "partially Closed" states. Consequently, it was revealed that the most crystal structures containing two nucleotide-bound β subunits represent the pre-hydrolysis state. Moreover, ATP-waiting state was found to correspond to a novel combination of conformation. Thus cooperative domain motions of three β subunits drive rotation of the central stalk subunit γ.

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S1.41 Single molecule analysis of the tentoxin-induced molecular processes of inhibition and stimulation of CF_1

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Tentoxin, a cyclic peptide produced by phytopathogenic fungi inhibits the chloroplast F_1-ATPase in sensitive plants at nanomolar to micromolar concentrations, whereas higher concentrations of tentoxin stimulate the activity up to 2–10 folds. By using the cyanobacterial F_1-ATPase obtained from *Thermosynechococcus elongates* BP-1 as a model enzyme, we analyzed both the inhibition and stimulation effects of tentoxin at a single molecule level, and successfully clarified the molecular steps involved in both processes. Inactivation delays the dwell time of a single step in the complete 360° turn of the γ subunit. Consequently the enzyme shows an asymmetric rotation. In contrast, rotation in stimulated F_1 particle is smooth and accompanied by strongly reduced ADP inhibition. Based on these observations and the kinetic analyses of the enzyme activity in the presence of tentoxin, the molecular processes of inhibition and stimulation of CF_1-type ATPase by tentoxin are discussed.

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S1.42 H^+-ATP synthase from chloroplasts: Rotational movement of subunits in single enzymes during ATP synthesis

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The ATPsynthase from chloroplasts forms ATP from ADP and phosphate. This energy-consuming reaction is driven by a transmembrane electrochemical potential difference of protons. Rotation of the γ subunit during ATP synthesis has been shown for *E. coli* ATPsynthase. Similar studies with a eukaryotic ATPsynthase during ATP synthesis have not been reported yet. In order to observe single enzymes we labelled the rotor-subunit γ at the cysteine C322 with ATTO532. An acceptor fluorophore, ATTO-655-AMPPNP, was introduced via substrate-enzyme-interaction at the so-called non-exchangeable ATP binding site. This procedure is necessary because cysteine mutants are not readily available for eukaryotic enzymes. Intermolecular FRET measurements of single double labelled enzymes reconstituted into liposomes were carried out with a home-built confocal microscope. During non-catalytic conditions we observed photon bursts with a constant FRET efficiency. During ATP synthesis bursts with changing FRET efficiencies were found indicating a distinct step-wise repeating sequence of FRET-levels (1-2-3-1-…). This corresponds to a 120° stepwise rotation of the γ-subunit relative to the non-exchangeable ATP binding site on the α-subunit.

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S1.43 Dimeric ATP synthase in thylakoid membranes of green algae and cyanobacteria

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To enlighten the supramolecular organisation of H^+-ATP synthases, native electrophoresis is the method of choice. Fundamental protein interactions are preserved during this kind of electrophoresis demonstrating the presence of stoichiometric supramolecular assemblies. While dimers and higher oligomers have been frequently described for the mitochondrial ATP synthase in biochemical and structural investigations, ATP synthase dimers in thylakoid membranes are much less characterised. In the chloroplasts of the green algae *Chlamydomonas reinhardtii*, we demonstrated the existence of such ATP synthase dimers. In contrast to the mitochondrial ones, the plastidic dimers dissociate upon addition of phosphate. The influence of this ATP synthase substrate on the supramolecular organisation suggested a physiological role of dimerisation. We could demonstrate that the dimer to monomer ratio of chloroplast ATP synthase is altered by the growth conditions. By labelling with stable isotopes, an increased assembly of ATP synthase dimers on the expense of pre-existing monomers during photomixotrophic growth compared to a photoautotrophic culture was observed. Our recent studies reveal the presence of ATP synthase dimers not only in *C. reinhardtii*, but also in thylakoid membranes of other alga and even in cyanobacteria. Our data demonstrate that dimers of chloroplast ATP synthases have an important physiological role and are common over a broad range of thylakoid containing organisms.

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S1.44 Mass determination of membrane protein complexes in detergent solution: The c rings from F-ATP synthases

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F_1F_o-ATP synthases are bipartite molecular motors able to convert an electrical membrane potential into ATP by a rotational