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