conformations of the various subunits in the F_1-complexes have been influenced by lattice contacts between F_1-complexes in the crystals. The conformations of the subunits in the $\alpha_3\beta_3$-domains are influenced little, if at all, by crystal contacts. Therefore, the interpretation of the conformations of these subunits as representing intermediates in the catalytic cycle is valid. Only when the crystals were highly dehydrated to decrease the dimensions of the unit cell, packing the F_1-complexes more closely in the crystal lattice, was any change apparent: there was a slight inward movement of the C-terminal helices of α-subunits. As has been noted many times in the past [3, 4], crystal contacts often influence the conformation of the central stalk significantly, and so the interpretation of the position that its exposed foot has adopted in the crystal structures, in relation to the rotary cycle of the central stalk, has to be carried out with caution.

References

[1] Abrahams JP, Leslie AG, Lutter R, Walker JE (1994) *Nature* **370:** 621–628.
[2] Bowler MW, Montgomery MG, Leslie AG, Walker JE (2007) *J. Biol. Chem.* **282:** 14238–14242.
[3] Menz RI, Walker JE, Leslie AG (2001) *Cell* **106:** 331–341.
[4] Gibbons C, Montgomery MG, Leslie AG, Walker JE (2000) *Nat. Struct. Biol.* **7:** 1055–1061.

doi:10.1016/j.bbabio.2010.04.100

2P.4 Binding of the inhibitor proteins IF_1 to mitochondrial F_1-ATPases

John V. Bason, Graham C. Robinson, Michael J. Runswick, Ian M. Fearnley, John E. Walker
Medical Research Council, Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge, CB2 0XY, UK
E-mail: walker@mrc-mbu.cam.ac.uk

In the structures of the complexes of bovine F_1-ATPase with residues 1–60 of bovine $IF_{1\ [1]}$, and of the yeast F_1-ATPase with residues 1–53 of yeast $IF_{1\ [2]}$, a long α-helix in the inhibitor proteins is bound in a deep groove at a catalytic interface between the C-terminal domains of the β_{DP}- and α_{DP}-subunits. In order to assess the contributions of specific amino acids in bovine IF_1 to binding, point mutations have been introduced singly throughout the long α-helix, and the effects on inhibitory properties have been measured. These experiments show that bovine IF_1 is bound mainly *via* hydrophobic interactions between its long α-helix with the C-terminal domain of β_{DP}-subunit, and in one case with the β_{TP}-subunit. In addition, there is a significant salt bridge between residue E30 in the inhibitor and residue R408 in the β_{DP}-subunit. Yeast IF_1 is bound in a similar way, but in the long α-helix there are significant local differences. The inhibitors also differ in the way that their N-terminal regions bind to F_1-ATPase. Residues 14–18 of bovine IF_1 form a short α-helix that interacts with the γ-subunit in the central stalk of the enzyme, whereas the equivalent region of yeast IF_1 has an extended loop structure that forms a salt bridge network with the γ- and α_E-subunits. Bovine IF_1 is a more potent inhibitor than yeast IF_1. The K_i values are: bovine IF_1 1–60 with F_1-ATPase 29.8 nM^{-1}, and with yeast F_1-ATPase 7.1 nM^{-1}; yeast IF_1 (E21A) with yeast F_1-ATPase, 16.0 nM^{-1}, and with bovine F_1-ATPase 217.5 nM^{-1}.

References

[1] Gledhill JR, Montgomery MG, Leslie AG, Walker JE (2007) *Proc. Natl. Acad. Sci. U. S. A.* **104:** 15671–15676.
[2] Robinson GC, Montgomery MG, Mueller DM, Leslie AGW, Walker JE. (In preparation).
[3] Bason JV, Runswick MJ, Fearnley IM, Walker JE (2010) *J. Mol. Biol.* (In press).

doi:10.1016/j.bbabio.2010.04.101

2P.5 Combination of single molecule FRET spectroscopy with optical tweezers: A powerful tool for mechanistic studies of enzymes

Roland Bienert, Peter Gräber
Department of Physical Chemistry, Albert-Ludwigs-University of Freiburg, Albertstrasse 23a 79104 Freiburg, Germany
E-mail: roland.bienert@physchem.uni-freiburg.de

The H^+-ATP synthase forms ATP, the energy currency of the cell, from ADP and phosphate. This energy-consuming reaction is driven by a transmembrane electrochemical potential difference of protons. The H^+-ATP synthase has been labelled by two fluorophores which allow fluorescence resonance energy transfer (FRET). The FRET efficiency strongly depends on the distance between the fluorophores. This effect can be used to measure distances and changes in distances between the labelled subunits of the protein. The H^+-ATP synthase is reconstituted into liposomes and fluorescence bursts are observed when a single proteoliposomes traverses the detection volume of the confocal microscope. During the burst (duration 100 ms on average) FRET and FRET changes can be observed. This time is often too short to observe a full catalytic cycle. To increase the detection time we trap a single proteoliposome with an optical trap exactly in the centre of the confocal detection volume, so the duration of the burst is not controlled by diffusion of the proteoliposome. By this approach we obtain longer observation times, which allow a detailed analysis of intramolecular movements of subunits. With this combination of optical tweezers and single molecule fluorescence it is possible to investigate the mechanism of membrane integrated or associated proteins in a nature-like environment in long-time studies and the problem arising from immobilisation of the enzyme is avoided.

doi:10.1016/j.bbabio.2010.04.102

2P.6 Single molecule spectroscopy of membrane bound H^+-ATP synthases from chloroplasts

Markus Burger, Roland Bienert, Peter Gräber
Institut für Physikalische Chemie, Albert-Ludwigs Universität Freiburg, Germany
E-mail: markus.burger@physchem.uni-freiburg.de

Subunit movements within the H^+-ATP synthase from chloroplasts ($CFOF_1$) are investigated by single molecule spectroscopy during ATP synthesis. The γ-subunit is covalently labeled at the disulfide bond between γC199 and γC205 with a fluorescence donor (ATTO532). A fluorescence acceptor (ATP-ATTO665) is non-covalently bound to a non-catalytic site at one α-subunit. The donor and acceptor labeled $CFOF_1$ is integrated into the liposomes and a transmembrane pH-difference is generated by an acid base transition. Single-pair fluorescence resonance energy transfer is measured in freely diffusing proteoliposomes with a confocal two channel microscope. The fluorescence time traces reveal a repetitive three stepped rotation of the γ-subunit relative to the α-subunit during ATP synthesis. During catalysis the central stalk interacts, with equal probability, with each αβ-pair. Without catalysis the central stalk interacts with only one specific αβ-pair and no stepping between FRET levels is observed.

doi:10.1016/j.bbabio.2010.04.103