The divalent metal binding sites of beef heart mitochondria F1ATPase were studied by FT-ESEEM spectroscopy, using Mn(II) as a paramagnetic probe, which replaces the naturally occurring Mg(II) and maintains the enzyme catalytic activity. Purified F1ATPase still containing three endogenous tightly bound nucleotides, named MF1 (1,2), was obtained under mild conditions, whereas a harsher treatment gave a fully nucleotide depleted enzyme, named MF1 (0,0). When MF1(1,2) was loaded with Mn(II) in 1:0.8 ratio, the spectrum showed evidence of a nitrogen interacting with the metal, while this interaction was not present in the spectrum of MF1(0,0) loaded with Mn(II) in the same ratio. However, when MF1(0,0) was loaded with 2.4 Mn(II), the spectrum showed metal-nitrogen interaction resembling that of MF1(1,2) loaded with Mn(II) in 1:0.8 ratio. When MF1(1,2) was loaded with 2.4 Mn(II) the metal-nitrogen interaction signal remained and a phosphorous coordination to the metal was also evident, indicating a binding of Mn^{2+} to a site containing a tightly bound nucleotide but metal free. These results strongly support the role of the metal alone in structuring the catalytic sites of the enzyme while ESEEM technique appears to be a sensitive and suitable spectroscopic method for conformational studies of MF1 with the advantage of using proteins in frozen solution.

doi:10.1016/j.bbabio.2008.05.058

S1.21 Thermodynamic constraints in the reversal of adenine nucleotide translocase during the reversal of F0–F1 ATP synthase caused by respiratory chain inhibition: Critical role of substrate-level phosphorylation

Christos Chinopoulos[a], Miklos Mandi[a], Katalin Takacs[a], Laszlo Csanady[a], Akos A. Gerencser[b], Lilla Turiak[a], Vera Adam-Vizi[a]
[a]*Semmelweis University, Department of Medical Biochemistry, Budapest, Hungary*
[b]*Buck Institute, Novato, USA*
E-mail: cch@puskin.sote.hu

Mitochondria are the main ATP producers in the cell. However, during respiratory chain inhibition mitochondria become ATP consumers due to reversal of the F0–F1 ATP synthase. The current belief is that under these conditions, extramitochondrial ATP is carried into the matrix chiefly through the reversal of the ANT. For this, the mitochondrial membrane potential must reach values more positive than both the reversal potential of the ATP synthase (Erev-ATPase) and that of the ANT (Erev-ANT). Here we show that in mitochondria capable of substrate-level phosphorylation, inhibition of the respiratory chain shifts the membrane potential to a range bracketed by the Erev-ATPase and the Erev-ANT, the latter being more negative than the former. As a consequence of this, reversal of the ATP synthase generates a sufficient membrane potential to oppose the ANT from operating in reverse mode, for as long as substrate-level phosphorylation is maintained. During respiratory chain inhibition the ANT can only be reversed by a concomitant uncoupling when the membrane potential attains values more positive than the Erev-ANT, or by incapacitating substrate-level phosphorylation. The latter maneuver shifts the Erev-ANT towards more negative values than the prevailing mitochondrial membrane potential.

doi:10.1016/j.bbabio.2008.05.059

S1.22 Determination of unidirectional H^+ transport in F-type ATPases

Alexander Wiedenmann, Peter Dimroth, Christoph von Ballmoos
Institute of Microbiology, ETH Zurich, Switzerland
E-mail: wiedenmann@micro.biol.ethz.ch

F-type ATP synthases are ubiquitous enzymes, which can either synthesize ATP using an electrochemical gradient or hydrolyze ATP to generate a membrane potential. We have investigated the properties of these two working modes in the Na^+ dependent enzyme from *Propionigenium modestum* and the H^+-dependent enzyme from *E. coli*. We found that ΔpNa and $\Delta\psi$ are required for efficient ATP synthesis in the Na^+-dependent enzyme. In accordance with this, we were able to show that both driving forces are capable of energizing Na^+-transport through F_0, when the enzyme was driven in synthesis direction. Interestingly, ΔpNa alone could not stimulate ion transport in the reverse direction as found during hydrolyis, whereas a $\Delta\psi$ served efficiently as driving force. The two directions are therefore kinetically not equivalent. Furthermore, we observed different K_D values for Na^+ for either transport direction. Whereas the K_D for Na^+ in hydrolysis direction is in the range of 1 mM, it was determined to be 15 mM during ATP synthesis. To test whether this asymmetry is present in H^+ dependent ATP syntheses as well, we developed a fluorescent assay for monitoring transport in either synthesis direction or in hydrolysis direction through the F_0 part of *E. coli* and chloroplast ATP synthase. The assay allowed quantitative determination of initial transport rates for H^+ dependent ATP synthases from different organisms in a well defined *in vitro* system.

doi:10.1016/j.bbabio.2008.05.060

S1.23 The H^+/ATP ratio of H^+-ATPsynthases from chloroplasts, *E. coli* and mitochondria

Jan Petersen[a], Paola Turina[b], Peter Gräber[a]
[a]*Department of Physical Chemistry, University of Freiburg, Germany*
[b]*Department of Biology, University of Bologna, Italy*
E-mail: peter.graeber@physchem.uni-freiburg.de

The H^+/ATP ratio is an important parameter for energy balance in cells and for the mechanism of coupling between proton transport and ATP synthesis. Rotational catalysis predicts that the H^+/ATP coincides with the ratio of the c-subunits to the β-subunits, implying that a value of 4.7 is expected in the chloroplast ATPsynthase and a value of 3.3 is expected in the mitochondria and *Escherichia coli* enzyme. This ratio can be determined based on the energetics given by the chemiosmotic theory. The isolated enzymes were reconstituted into liposomes. The internal phase of the liposomes was equilibrated with the acidic medium during reconstitution, so that the internal pH could be measured with a glass electrode. An acid-base transition was carried out and the initial rates of ATP synthesis or ATP hydrolysis were measured with luciferin/luciferase as a function of ΔpH at constant $Q=[ATP]/([ADP][P_i])$. From the shift of the equilibrium ΔpH as a function of Q, the standard Gibbs free energy for phosphorylation and the H^+/ATP ratios were determined.

doi:10.1016/j.bbabio.2008.05.061

S1.24 Effect of Pi and ADP on the intrinsic uncoupling in the isolated and reconstituted ATPsynthase of *E. coli*

Manuela D'Alessandro, Paola Turina, B. Andrea Melandri
Department of Biology, Laboratory of Biochemistry and Biophysics, University of Bologna, 40126 Bologna, Italy
E-mail: paola.turina@unibo.it