2P.7 F_1F_O ATP synthase mutants in *Chlamydomonas*: Stability and oligomycin resistance mediated by atypical Asa7 protein; interaction between chloroplastic and mitochondrial bioenergetics

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In yeast, mammals, and land plants, F_1F_O ATP synthase (complex V) comprises about 15 conserved subunits. In this work, we show that complex V from chlorophycean green algae has an atypical subunit composition of its peripheral stator and dimerization module, with 9 subunits of unknown evolutionary origin (Asa subunits), while complex V has a canonical subunit composition in other classes of green algae. In addition, growth, respiration and ATP levels in Chlorophyceae are also barely affected by oligomycin concentrations that affect representatives of the other classes of Chlorophytes. We then isolated *Chlamydomonas* mutants lacking either beta subunit (Atp2) or an atypical subunit (Asa7). The Atp2 mutant is an obligate phototroph lacking complex V assembly and ATP synthesis coupled to the respiration. In addition, Atp2-deficient mitochondria are deprived of cristae, and rearrangements of the photosynthetic apparatus and thylakoid organization are observed. In contrast, the loss of Asa7 subunit has no impact on cell bioenergetics or organelles structures, but it destabilizes the enzyme dimeric form *in vitro* and renders growth, respiration and ATP level sensitive to oligomycin. Altogether, our results suggest that the loss of canonical components of the stator happened at the root of chlorophycean lineage and was accompanied by the recruitment of novel polypeptides. Such a massive modification of stator features might have conferred novel properties, including the stabilization of the enzyme dimeric form and the shielding of the proton channel. Our study also contributes to the understanding of the yet poorly-studied bioenergetic interactions between organelles in photosynthetic organisms.

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2P.8 Purification and characterisation of F-ATPase from three species of fungi

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F-ATPase has been purified from mitochondria from *Pichia angusta*, *Pichia pastoris* and *Yarrowia lipolytica* by affinity chromatography with a monomeric pH insensitive form of the bovine yeast inhibitor protein, IF_1. The subunit compositions of the complexes have been characterized by SDS-PAGE, by mass spectrometric analysis of tryptic peptides, and by measurement of the masses of the subunits by LC-MS [1]. The impact of phospholipids on specific activities and the sensitivity to oligomycin of the complexes has been studied. The enzymes have been reconstituted in phospholipid vesicles and proton pumping was measured with the pH sensitive probe amino-6-chloro-2-methoxy-acridine (ACMA). ACMA quenching was observed after addition of ATP, halted by addition of oligomycin and reversed by addition of the ionophore gramicidin. The thermostability of the purified complexes was investigated by following the ATP hydrolysis activity from 25 °C to 70 °C. As expected, the *P. angusta* enzyme was the most thermostable. It has been selected for crystallisation trials.

Reference

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2P.9 3-Iodothyronamine favours IF_1 release from F_OF_1 ATP synthase

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F_OF_1 ATP synthase has been demonstrated as a good molecular target for drugs in the treatment of various diseases and regulation of energy metabolism so that the development of new F_OF_1 ATP synthase-directed agents is encouraged nowadays. On the basis of a structural similarity with well characterised enzyme inhibitors, such as resveratrol, the inhibitory capacity of 3-iodothyronamine (T_1AM), a naturally occurring derivative of thyroid hormone, has been investigated [1]. First, T_1AM effect on the activity of the mitochondrial respiratory chain was excluded. Then, T_1AM direct inhibitory effect on ATP synthase/ase activity has been investigated on F_OF_1 whole enzyme using preparations of submitochondrial particles (SMP) as model. When $\Delta\Psi$ is sustained by succinate, IC_{50} values indicate that T_1AM has an inhibitory potency on ATP synthase activity similar to that of resveratrol. On the contrary, when $\Delta\Psi$ is inhibited by rotenone and the enzyme can only hydrolyze ATP, T_1AM shows an activating effect at a low concentration (10–50 μM) followed by a limited inhibitory effect at a higher concentration (50–150 μM). The activating action results more marked on SMP preparations having a higher content of the natural inhibitor protein (IF_1) [2], while it disappears on IF_1-free AS particles. Such effect is consistent with IF_1 release from the enzyme as a result of T_1AM binding. To support this evidence, immunoblotting analysis was performed on high-IF_1 containing SMP after treatment with T_1AM. The dose–response curve shows that IF_1 release occurs starting from the nanomolar T_1AM range suggesting that this mechanism could be of physiologic relevance and distinct from the inhibitory one. To help into the indirect localization of T_1AM inhibitory binding site, purified IF_1–free F_1 was used as a model. Kinetic analysis shows that T_1AM exerts a mixed type inhibition of ATPase activity as resveratrol does with a similar inhibitory potency. Nevertheless, competition experiments show that the inhibitory effects of T_1AM and resveratrol are additive indicating that the binding of the two inhibitors to F_1 is not mutually exclusive. This suggests distinct binding sites for the two drugs in agreement with peculiar T_1AM action. The binding site/s of T_1AM is/are now under investigation by further kinetic analyses and by molecular modelling with reference to available F_1 native and inhibited structures.

References

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