

Comparisson of various methods for measurement of reactive oxygen species production in fibroblasts from patients with deficiencies in ATP synthase

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Deficiencies in enzymes of oxidative phosphorylation (OXPHOS) cause a broad range of mitochondrial encephalomyopathies. Apart from decrease of ATP production, elevated levels of reactive oxygen species produced by defective OXPHOS complexes have been implicated as an important pathological mechanism of mitochondrial diseases [1]. Recently, it was proposed that elevated ROS production is present also in cells with deficiency in ATP synthase of either mitochondrial [2] or nuclear [3] origin. Presumably, high levels of mitochondrial membrane potential ($\Delta\Psi_m$) in such cells represent an important prerequisite for increase in ROS production.

In this work we used fibroblasts derived from patients with deficiencies in ATP synthase to study *in vivo* ROS production and its influence by uncouplers or inhibitors of respiratory chain complexes.

We established methods for measurement of ROS production (with carboxy-H₂DCFDA, using either single- or two-photon excitation) and changes in $\Delta\Psi_m$ (with TMRM) on confocal microscope (Leica TCS SP 2) with the possibility to relate both signals to mitochondrial mass, determined by mitochondria selective probe MitoTracker DeepRed. For determination of ROS production on intact cells we also used fluorescence plate-reader (Wallac Victor²), where we correlated the ROS production to protein- or DNA-content in individual wells. We tried to compare data on ROS production obtained by these three methods with respect to absolute and relative values of ROS production and the intensity of probe autooxidation and photoactivation. Furthermore we studied effect of various uncouplers (FCCP, CCCP, valinomycin) and inhibitors of respiratory chain (Antimycin A, Rotenone).

We found that patient cells produce significantly higher amounts of ROS than controls. Our data further support the importance of high mitochondrial membrane potential ($\Delta\Psi_m$) caused by defective discharge of proton gradient by ATP synthase for increased ROS production in patient cells as pretreatment with uncoupler causes decrease to levels close to control cells.

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