11L.3 Mitochondrial respiration and membrane potential are regulated by the allosteric ATP-inhibition of cytochrome *c* oxidase

Bernhard Kadenbach[1], Rabia Ramzan[1],
Katrin Staniek[2], Sebastian Vogt[1]
[1]*Biomedical Research Center, Philipps-University, D-35032 Marburg, Germany*
[2]*Biochemical Pharmakology and Toxicology, Veterinary-Medical University of Vienna, Austria*
E-mail: kadenbach@staff.uniarburg.de

The problems of measuring the allosteric ATP-inhibition of cytochrome *c* oxidase (CcO) in isolated mitochondria are described. Only by increasing the ATP/ADP ratio with the ATP-regenerating system phosphoenolpyruvate and pyruvate kinase to high values full ATP-inhibition of CcO could be seen by kinetic measurements. The mechanism was proposed to keep the mitochondrial membrane potential ($\Delta\Psi_m$) at low values when matrix ATP/ADP ratios are high, as found in living cells and tissues (100–140 mV). In contrast, with isolated mitochondria high $\Delta\Psi_m$ values are generally obtained (180–220 mV). We have measured $\Delta\Psi_m$ in isolated rat liver mitochondria by using a TPP^+-electrode. With glutamate plus malate as substrates $\Delta\Psi_m$ decreased from 233 to 123 mV after addition of phosphoenolpyruvate and pyruvate kinase, which was reversed to the original $\Delta\Psi_m$ after addition of ATP. The decrease of $\Delta\Psi_m$ is explained by reversal of the gluconeogenetic enzymes pyruvate carboxylase and PEP carboxykinase yielding ATP and GTP, thus increasing the matrix ATP/ADP ratio. With rat heart mitochondria, which lack these enzymes, no decrease of $\Delta\Psi_m$ was found. From the data we conclude that in vivo high ATP/ADP ratios keep $\Delta\Psi_m$ at low values by the allosteric ATP inhibition of CcO, thus preventing the generation of ROS and formation of degenerative diseases. It is proposed that respiration in living eukaryotic organisms is mainly controlled by the allosteric ATP-inhibition of CcO, and not — as generally assumed — by 'respiratory control' according to the Mitchell Theory.

doi:10.1016/j.bbabio.2010.04.277

11L.4 Cytochrome *c* oxidase: Electrogenic mechanism and regulation by calcium ions

Alexander A. Konstantinov
Moscow State University, A.N.Belozersky Institute of Physico-Chemical Biology, Russia
E-mail: konst@genebee.msu.su

Electrogenic mechanism of CcO. Time-resolved assays of the **F→O** transition of bacterial CcO induced by single-electron photoreduction of Compound **F** reveal 4 basic electrogenic processes: (1) vectorial e^- transfer from Cu_A to heme *a*, followed immediately by (2) intraprotein proton displacement preceding e^- transfer to the binuclear site; (3) translocation of the pumped and (4) of the chemical protons. Time-resolved measurements of $\Delta\psi$ generation by mutant CcO with replacement in the D-proton channel reveal drastic discrepancy between behaviour of the **F→O** transition of the enzyme during oxidation of the fully reduced oxidase in the so-called flow-flash studies, and the same step induced by photoinjection of a single e^- into Compound **F**. Presumably, during oxidation of the fully reduced enzyme, the oxidase can miss uptake of at least 1 proton, which entails formation of an abnormal deprotonated oxidized form "$\mathbf{O}_H$". This abnormal species refills the proton vacancy upon its reduction by the 1st electron in the next turnover. *Direct regulation of CcO by calcium ions.* Mitochondrial CcO binds Ca^{2+} reversibly at a specific site at the outer face of the membrane close to heme *a*. Ca^{2+} binding brings about a red shift of the absorption spectrum of heme *a* in the reduced or oxidized state which allows to determine accurately characteristics of the binding. In bovine heart CcO, K_d for Ca binding is close to 1 μM. Disappointedly, Ca binding with CcO was not found earlier to affect any functional characteristics of CcO. We now show that at typical physiological rates of electron transfer (about 10 s^{-1}), Ca^{2+} inhibits isolated bovine CcO by about 60% with K_i very close to K_d of its binding with the enzyme. At the same time, no effect of Ca^{2+} is observed under usual CcO activity assays (TN > 100 s^{-1}). The inhibition is observed with cytochrome c^{2+} or artificial electron donors to CcO and is Ca^{2+}-specific. The Ca-induced inhibition of CcO has been demonstrated with the intact mitoplasts (in the presence of the uncoupler) from several tissues. The extent of inhibition is tissue specific, reaching 80–90% with liver mitochondria. Physiological role of the calcium-induced effect will be discussed.

doi:10.1016/j.bbabio.2010.04.278

11L.5 Structural and functional characterisation of alternative oxidases: Role of conserved tyrosines in the catalytic cycle

Mary S. Albury[1], Catherine Elliott[1], Yasutoshi Kido[2],
Kiyoshi Kita[2], Anthony L. Moore[1]
[1]*Biochemistry and Biomedical Sciences, School of Life Sciences, University of Sussex, Falmer, Brighton, UK*
[2]*Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan*
E-mail: a.l.moore@sussex.ac.uk

Alternative oxidases are not only found in plants, but also occur in many fungi as well as several pathogenic organisms including the blood parasite *Trypanosoma brucei* and the intestinal parasite *Cryptosporidium parvum*. Because of their absence in the mammalian host, AOX proteins are therefore considered to be important potential therapeutic targets in these systems. To date, no high-resolution AOX structure has been determined although we have recently obtained diffracting crystals from the trypanosomal alternative oxidase at a resolution greater than 2.9 Å. The AOX is an integral (about 32 kDa) interfacial membrane protein that interacts with a single leaflet of the lipid bilayer, and contains a non-haem diiron carboxylate active site. Certain amino acids have been implicated in Q-binding, because of their influence on the sensitivity to AOX inhibitors. We have previously suggested that the AOX reaction cycle may involve amino acid radicals, and close scrutiny reveals there are only a few fully conserved residues that could perform such a role (Tyr-253, Tyr-266, Tyr-275, Tyr-299 and Trp-206). Of the four tyrosine residues, we have previously demonstrated that only the hydroxyl moiety of Tyr-275 is essential for AOX activity and therefore likely to be of catalytic importance. In this talk, we report on the finding that mutagenesis of Trp-206 to phenylalanine or tyrosine fully abolishes AOX activity in contrast to that observed with either Tyr-253 or 299 both of which retained partial activity. Interestingly the Y266A mutant, which is not fully conserved across all organisms and hence its role in the catalytic cycle is uncertain, inhibits respiratory activity by approximately 90%. Of our other mutants that retain measurable activity, none appear to influence AOX sensitivity to Q-like inhibitors, however, the mutation of Thr-179 or Cys-172 to alanine results in a significant increase in the apparent affinity of AOX for O_2. The significance of these results on the mechanism by which alternative oxidases reduce oxygen through to H_2O will be discussed in detail.

doi:10.1016/j.bbabio.2010.04.279